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W. B. BRIERLEY

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C. T. GIMINGHAM

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THE INTERNAL TEMPERATURES OF FRUIT-TREE BUDS. II

BY JOHN GRAINGER, PH.D., B.SC.

Tolson Memorial Museum, Ravensknowle, Huddersfield

(With 10 Text-figures)

CONTENTS

INTRODUCTION

THE present paper amplifies and extends a previous investigation (Grainger & Allen, 1936) where temperatures within dormant buds of apple, black currant and raspberry were measured by thermo-electric apparatus.

METHODS

A circuit of two fine iron-constantan thermocouples connected in series was used to make continuous measurements of temperature differences at the junctions upon a Cambridge thread-recording galvanometer. Details were given in a previous paper (Grainger & Allen, 1936), and the records show actual air temperatures, but only compare internal bud temperatures with those of the surrounding air. Atmospheric temperatures were occasionally measured by a standard bimetallic thermograph made by Messrs Negretti and Zambra. This was placed near to the buds under investigation, and was enclosed within a louvre screen $18 \times 15 \times 14$ in. high. Thermo-electric circuits were further modified, in some of the experiments here described, to estimate solar radiation (Fig. 1) and "wet-bulb" temperatures (Fig. 2). Curves representing solar radiation occasionally went below zero on the chart at night, indicating radiation from the apparatus to an open sky (lowest curve, Fig. 9).

Two records were sometimes made upon each chart of the galvanometer, and were separated by a mechanical method. Two inked threads of contrasting colour were brought alternately below the recording mechanism, whilst a two-way switch changed

the circuits. Each circuit was thus always recorded by a particular colour, and the circuits were chosen for their contrast. Air temperatures and bud temperatures contrasted well, or buds with different temperature responses, such as apple and raspberry. Both curves on one chart had the same zero, which was marked by the usual method of disconnecting the common lead to the galvanometer for a few minutes each day.

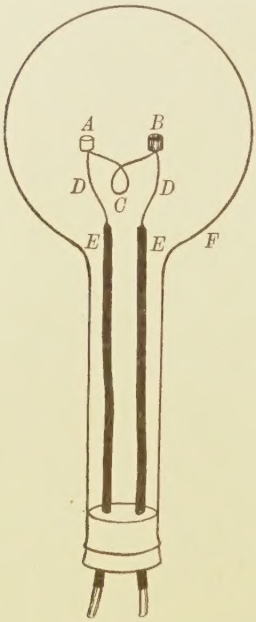

Fig. 1.

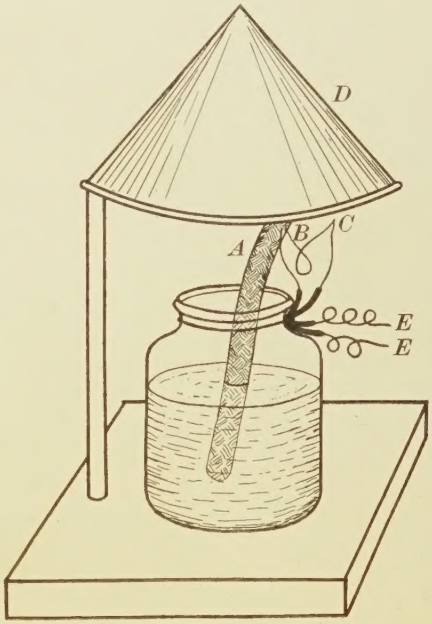

Fig. 2.

Fig. 1. Apparatus for the electrical measurement of solar radiation. *A*, a bright cylinder of brass; *B*, a dark cylinder; *C*, constantan wire; *D*, iron wire; *E*, leads of insulated copper wire; *F*, flask of Pyrex glass. Cylinder *A* reflected some of the sun's heat, whilst *B* absorbed it, thus giving a temperature difference which was recorded.

Fig. 2. Apparatus for the electrical measurement of humidity. *A*, wick dipping into water in the bottle; *B*, thermocouple inserted into the wick; *C*, thermocouple in the air; *D*, hood; *E*, leads of insulated copper wire.

Results

The following results are selected from a total of 594 daily records made in 1936, 1937 and 1938.

The effect of frosts upon apple-bud temperatures

Fourteen records of frosts during the dormant period were obtained in 1936 and 1937. They exhibit similar features to six more which were obtained in the earlier investigations in 1932 and 1933 (Grainger & Allen,

1936), in that the bud temperature coincided fairly closely with the air temperature during the more severe part of the frost. Fig. 3, which is typical, shows this from 1 a.m. to 6 a.m. The rise in the relative bud temperature between 6 a.m. and 7 a.m. was due to the warming effects of the sun's radiation, which first reached the bud soon after 6 a.m. A fall in the bud temperature relative to that of the air followed between 7 a.m. and 8 a.m., but this does not represent a fall in the *actual* temperature of the bud. This was increasing all the time from 6.30 a.m. to 8.30 a.m., as shown by the dotted line in the upper curve of Fig. 3.

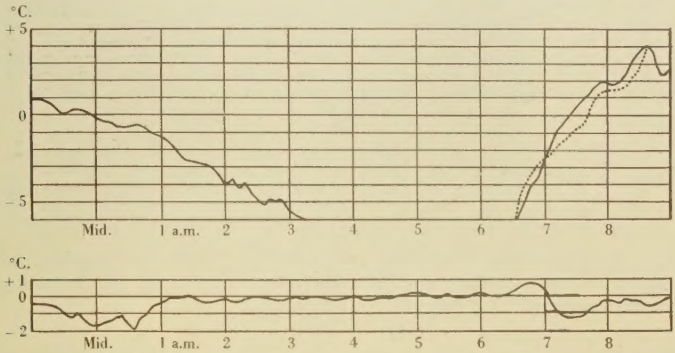

Fig. 3. A frost during dormancy of the apple bud. Upper curve, air temperature; lower curve, temperature of the bud in relation to the surrounding air. The actual temperature of the bud is shown by the dotted line in the upper diagram, between 6.30 a.m. and 8.30 a.m. 5–6 March 1937.

The early stages of the frost shown in Fig. 3 are marked by a rise in the temperature of the bud relative to the surrounding air between midnight and 1 a.m. This is somewhat comparable to the behaviour of opening buds to frosts (Grainger & Allen, 1936). Three more such records were obtained when buds were bursting in 1938, and Fig. 4 shows a typical example. The bud temperature approximated to that of the surrounding air until the latter reached freezing-point, but became relatively warmer when the air temperature fell slightly below that point, i.e. after midnight. This is the "frost compensation mechanism" (Grainger & Allen, 1936), but it is now obvious that it is not of great magnitude, and occurs only during slight frosts of 1 or 2° C., or at the commencement of more severe frosts. Though it is a frequent phenomenon, it cannot be of much practical significance.

It is to be expected that buds would have a different composition when they are opening from when they are dormant. The actual freezing-

points of standardized extracts were determined in 1937. Apple buds were not available in sufficiently large quantities, so hazel, oak and sallow (*Salix caprea*) were used. It is impracticable to obtain expressed sap from dormant buds unless inordinately large quantities are available, so a standardized extract was made with distilled water.

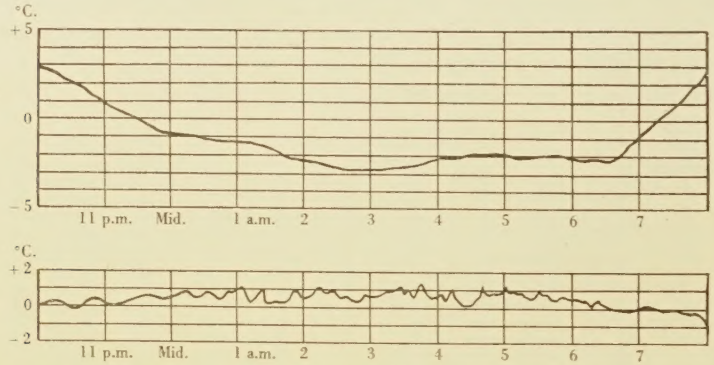

Fig. 4. A frost when buds of the apple (var. Allington Pippin) were bursting. Above, air temperature (bimetallic record). Below, temperature of the bud in relation to the surrounding air. 7–8 May 1938.

Freezing-points of such extracts were compared, immediately after their preparation, with the freezing-point of distilled water, by means of a Beckman thermometer. The percentages of water, of organic matter, and of ash were determined for portions of each sample of buds, and the differences recorded in the last two columns of Table I are of sufficient magnitude to be significant with relatively large variations in water content.

Table I. *Comparative estimations of standardized extracts from buds at various stages*

Tree	Condition of buds	Date	% water	% organic matter	% ash	Depression of freezing-point in °C.	Osmotic pressure in atm.
Hazel	Dormant	6. i	50·00	48·50	1·50	0·15	1·805
Hazel	Opening	19. iii	55·25	43·08	1·62	0·38	4·580
Hazel	Young leaves	5. v	71·40	25·83	1·46	0·11	1·327
Oak	Dormant	19. iii	45·52	52·00	1·98	0·13	1·568
Oak	Opening	5. v	69·20	28·46	2·31	0·17	2·050
Sallow	Dormant	19. iii	47·18	52·00	0·08	0·04	0·398
Sallow	Opening	5. v	71·54	26·51	1·74	0·10	1·206

The depression of the freezing-point of standardized extract is greatest at the time of opening of the buds. It is at this time also that the greatest

damage by frost can occur. Such damage, therefore, cannot be caused by any increased tendency of the sap to congeal at the time of opening, and other explanations must be sought.

The effect of frost control by orchard heaters upon bud temperatures

A practicable method of frost control by inexpensive heaters which burn crude oil has been introduced by Mr G. Harrington.[1] The question as to whether the heaters merely raised the temperature of the air, or heated the buds by direct radiation from the flames, was tested by ten trials in February 1937, and April and May 1938.

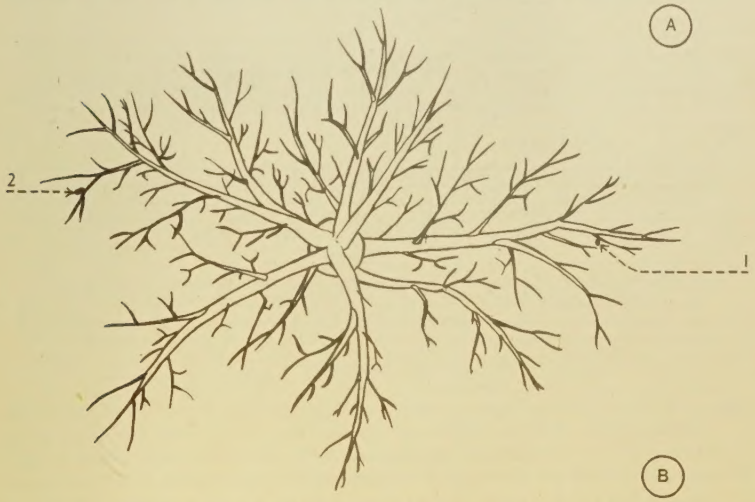

Fig. 5. Sketch plan of apple tree (var. Lord Suffield), showing position of the orchard heaters, A, B, and buds, 1, 2. Bud 1 could be heated by direct radiation from the flames of heater B, but was screened by the branch from A. Bud 2 was 8 ft. above the ground, and was not apparently affected by either of the heaters. 23 Feb. 1937.

Estimations during a radiation frost on the evening of 23 February 1937 provided typical results. Fig. 5 shows the arrangement of heaters round an apple tree of the variety Lord Suffield. Internal temperatures of two buds, 1 and 2 in the figure, were recorded, whilst air temperatures were measured by a screened thermograph placed close to bud 1, and by mercury thermometers round bud 2, and in the unheated parts of the orchard. Bud 1 was 2 ft. 6 in. above the ground, and 5 ft. from either

[1] Marketed by Messrs Geo. Monro, Ltd., of Waltham Cross.

heater; bud 2 was 8 ft. above the ground, and 16 ft. from either heater. The latter bud was quite unaffected by the heaters, for no difference could be found between temperatures of the air surrounding it and temperatures in the unheated parts of the orchard (see dotted line in I, Fig. 6). The apparent rise in the internal temperature of this bud (II in Fig. 6) is occasionally found with falling air temperature, and may be

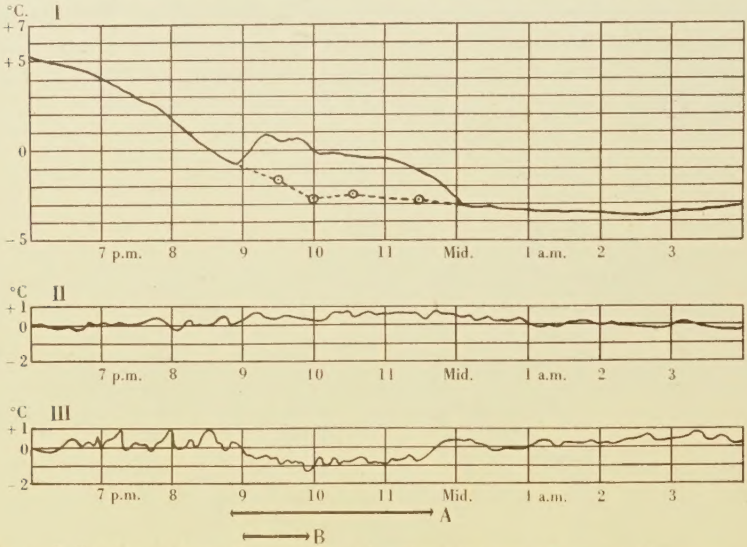

Fig. 6. I. Raising of the air temperature by orchard heaters 8.45 p.m. to 11.40 p.m.; bi-metallic record. Temperatures of the unheated air in the orchard are shown by the dotted line. II. Temperature (in relation to the surrounding air) of bud 2 (fig. 5) not affected by the heaters. III. Temperature of bud 1 (fig. 5) in relation to the surrounding air. A = length of time heater A was lit, namely 8.45 p.m. to 11.40 p.m. B = length of time heater B was lit, namely 9 p.m. to 9.50 p.m. Heater A could not heat bud 1 (curve III above) by direct radiation; heater B could possibly do so. 23 Feb. 1937.

due to delay of the bud in assuming the temperature of its surroundings. It gives the further proof of having no connexion with the heaters, that it continues for 1½ hr. after they are extinguished. Heating of the orchard under commercial conditions would, of course, have brought bud 2 within the sphere of two further heaters.

The greatest increase in air temperature accomplished by the heaters in any of the ten trials was only 3° C. (5·5° F.) when the nearest heater was 5 ft. from the thermograph. This only happened, moreover, when the air was still, or when a slight, downwardly flowing katabatic wind

(Cornford, 1937) impelled the warm air towards the thermograph. A rise of 2° C. (4° F.) was the usual increase in still air, but any greater distance than 5 ft. from heater to tree or thermograph quickly reduced the rise in temperature.

One heater (B in Fig. 5) was so disposed that it could possibly heat bud 1 by direct radiation, whilst the flames from the other heater (A) were shielded from the bud by a thick branch. Curve III in Fig. 6 shows the results, and indicates no difference between the effect of either heater or both. This means that the heaters exert their good effect by raising the temperature of the air, and not by direct radiation from the flames. The temperature of the bud was uniformly *lower* than the temperature of the

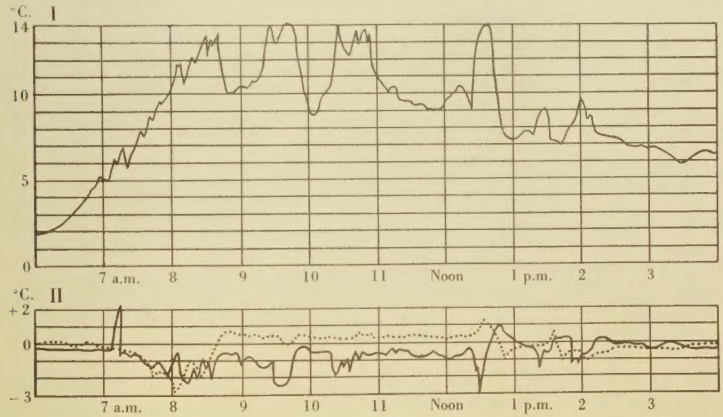

Fig. 7. I. Air temperature. II. Temperatures, in relation to the surrounding air, of two adjacent apple buds (var. Bramley's Seedling), 4 in. apart upon the same branch. 9 Oct. 1936.

surrounding air, owing to the effect of the heaters in decreasing the relative humidity of the air. Evaporation would thereby be increased, and the bud's temperature would therefore be lowered. Since the temperature inside a bud is lowered by heaters which only raise the air temperature 2 or 3° C., the margin of efficiency of the flame-type heaters would seem to be narrow. This aspect of the investigations is discussed more fully later in this paper.

Differences in temperature response between two adjacent apple buds

There is usually a strong agreement between the temperature reactions of two adjacent apple flower buds. Thirty-one pairs of records show such agreement, but Fig. 7, curve II, shows an instance where different

responses were observed between two flower buds separated by only 4 in. from each other. These two curves are typical of ten pairs of records. There is occasionally a qualitative similarity of the curves, but sufficient dissimilarity in detail to show some individuality of response by different buds exposed to approximately the same environment.

Temperature responses of the raspberry

Estimations of the temperatures of dormant raspberry buds, of opening raspberry flowers, of flowers and of fruits, were made upon the variety Lloyd George in 1936. Forty curves relating to the dormant

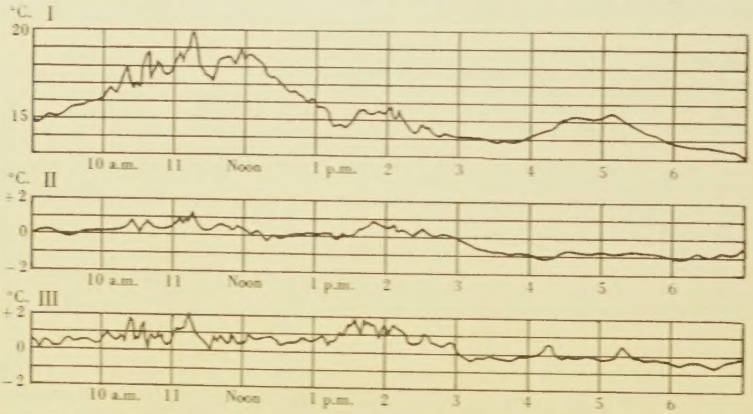

Fig. 8. I. Air temperature. II. Temperature of dormant apple bud (var. Bramley's Seedling) in relation to surrounding air. III. Temperature of dormant raspberry bud (var. Lloyd George) in relation to surrounding air. 17 Oct. 1936.

period show that the raspberry bud has a relation to air temperature similar to that possessed by the apple (Fig. 8). Both are warmed more than the air during sunshine, and are cooled below the air temperature at night, or when sunlight is diminished. The cooling is doubtless caused by evaporation, as was established for the apple (Grainger & Allen, 1936). Both apple and raspberry buds are occasionally cooled below the air temperature by a sudden burst of sunshine, acting through increase in evaporation, but the continuous effects of evaporation are not shown until the solar radiation diminishes (e.g. Fig. 8, II and III, 3–7 p.m.).

A simple experiment confirms the fact that water evaporates from raspberry buds. Twelve 6 in. pieces of dormant 1937 cane were separated into two equal groups. One group had the cut ends of the stem-pieces coated with vaseline, whilst the other group had all the buds coated in

addition. Each group was weighed, hung in the laboratory for seven days, and then reweighed. The group with untreated buds lost 19% of its original weight, whilst the vaseline coat upon buds of the other group reduced the loss in weight to 12%.

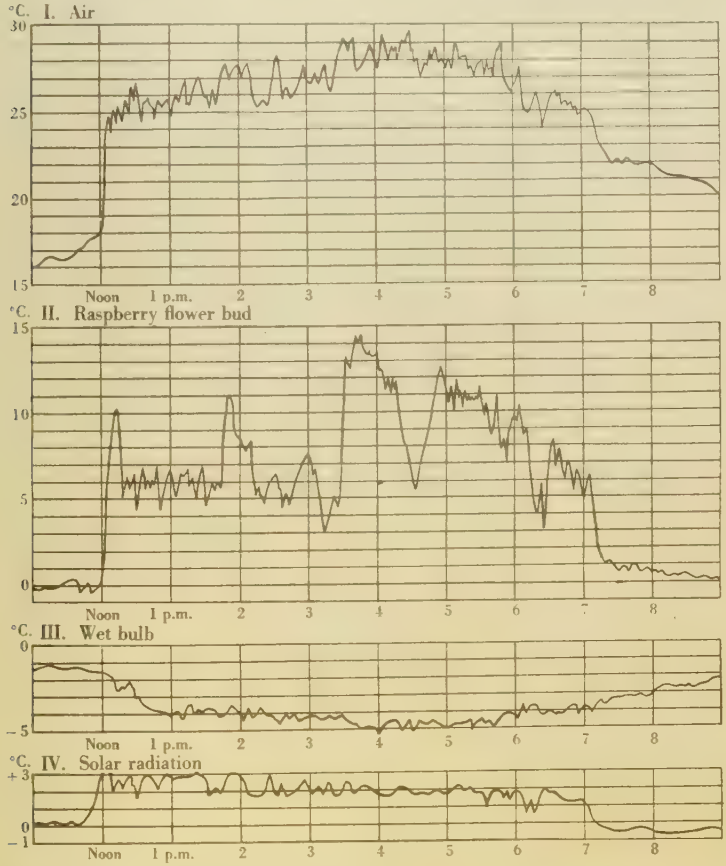

Fig. 9. I. Air temperature. II. Temperature of raspberry flower bud in relation to the surrounding air. III. Wet-bulb temperature. IV. Solar radiation. 20 June 1936.

Raspberry buds in the dormant state respond to rain as do apple buds (Grainger & Allen, 1936). There is a decrease in temperature, in relation to the air, whilst the rain is falling, but a quick recovery after it has ceased.

Raspberry flower buds, flowers, and developing fruits are all notable for the large amplitude of their temperature curves in sunshine. Fig. 9, which represents the temperature relations of an opening flower bud, is typical of all the stages mentioned above, and in all thirty-nine records have been taken. There is an obvious coincidence of the peaks of the bud-temperature curve with those of the air-temperature record, and

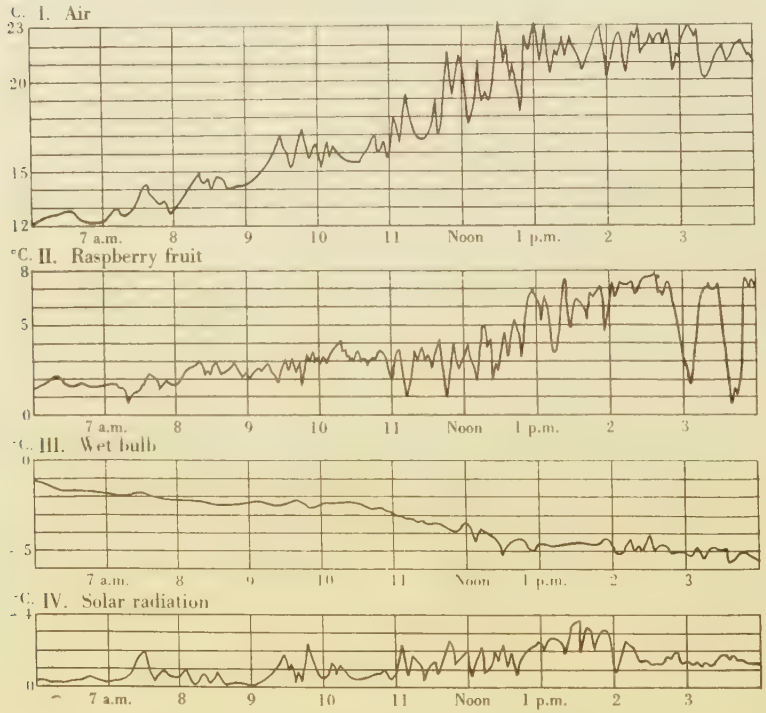

Fig. 10. I. Air temperature. II. Temperature of ripe raspberry fruit in relation to the surrounding air. III. Wet-bulb temperature. IV. Solar radiation. 16 July 1936.

also with the solar radiation maxima. Internal temperatures are often cooler than the surrounding air during the night. The rise in temperature of the ripe fruit during bright sunshine is less than with flower bud or flower (compare Figs. 9, 10), but is, however, greater than with the dormant bud. There are occasionally short periods, even during sunshine, when the fruit temperature descends below the temperature of the air, but the records are still very similar to those characteristic of the flower bud, flower and developing fruit.

The records of bud temperatures in the figures represent temperature *differences*. The curves must be added to those of the air temperatures for the same period, in order to represent the actual bud temperatures. The fact then emerges from Figs. 9 and 10 that the flower buds, flowers and fruits are often at a temperature of 40° C. (108° F.) during sunshine which only warms the air to 28° C. (81° F.). These figures are maxima, but they indicate the degree to which the small drupels can be warmed during sunshine, and probably help to explain the daily ripening of the raspberry crop which is so pronounced a feature of the soft-fruit industry. Table II shows that the raspberry has, moreover, the shortest period between the first flower and ripe fruit of any of the common species there listed. The information is compiled from averages for the years 1916–31 (Clark *et al.* 1933), and relates to Lowe's work in Worcestershire.

Table II. *Time in days between first flower and ripe fruit for several common trees and shrubs*

Alder, *Alnus glutinosa*	206
Apple, wild, *Pyrus malus*	157
Apple, cultivated, *Pyrus malus sativus*	126
Ash, common, *Fraxinus excelsior*	162
Ash, mountain, *Pyrus aucuparia*	90
Blackthorn, *Prunus spinosa*	156
Black currant, *Ribes nigrum*	73
Cherry, cultivated, *Cerasus vulgaris*	71
Elder, *Sambucus nigra*	91
Gooseberry, *Ribes grossularia*	95
Hawthorn, *Crataegus Oxyacantha*	110
Pear, *Pyrus communis*	157
Raspberry, *Rubus idaeus*	38

It is hoped to investigate further this suggested correlation between high internal temperature and quick ripening of the fruit.

Discussion and summary

1. The results here described follow a previous investigation (Grainger & Allen, 1936), where bud temperatures and air temperatures were recorded by thermo-electric methods. Humidity and solar radiation have also been recorded electrically in the present experiments.

2. Temperatures of apple buds show slight increases, in relation to air temperatures, during the early stages of a frost, particularly when the buds are bursting. This "frost compensation mechanism" is, however, very slight, and is of very little practical importance. Apple-bud temperatures during severe frosts in the dormant period usually show fairly close agreement with the air temperatures after the initial stages.

3. Experiments upon the control of frosts by the use of orchard

heaters burning crude oil show that their beneficial effects are due to warming of the air by convection, and not to heating of the bud by direct radiation. No difference in the bud-temperature record could be observed when the bud was exposed to, or shielded from, direct radiation from the flames. The internal temperature of an apple bud was uniformly lower than the temperature of the surrounding air during a frost in the dormant period, when orchard heaters were alight. Air temperatures round the bud were raised 2–3° C. above the surrounding air by the heaters.

Heaters with open flames appear to provide satisfactory control of frosts. The results here reported, however, raise the question as to whether a more efficient and economical type could not be evolved. Considerable heat must be lost by radiation from the flames to an open sky; lateral distribution of heat between heaters is not very good; and temperatures inside the bud are lowered by evaporation, owing to the drying effects of the heaters. It is suggested that a heater which would provide moist, heavy smoke would be a more efficient control of frosts than a flaming heater. Losses by radiation would be minimized, for smoke would have the additional advantage of providing insulation for the frost itself. Ground frosts or radiation frosts are caused by the radiation of heat from the earth to the heavens, and a blanket of smoke near the ground would diminish this loss. The inclusion of a small quantity of steam in the smoke would help to prevent the slight lowering of the bud's internal temperature by evaporation. Such a heating effect is provided by the practice of "smudging", where fires of partly dry refuse are used to provide moist smoke. The labour involved in building such fires is great, and an apparatus which would be as simple and cheap as the open flame oil heater, and yet would give a mixture of smoke and steam, should prove more efficient in practice. The heaters should also be disposed that any katabatic winds (Cornford, 1937) direct the heated air towards the trees. It would be somewhat easier to do this if lines of trees were planted diagonally across a slope, rather than straight up and down.

4. Two apple flower buds upon the same branch, and separated by only 4 in., may have divergent internal temperature relations, though a majority of records shows considerable agreement.

5. Temperature relations of raspberry buds, flowers and fruit have been studied. Dormant buds have internal temperatures which are similar to those of apple buds. They are warmed more than the air by solar radiation and are usually cooler than the air during the night, owing to evaporation from the buds.

Flower buds, flowers and developing raspberry fruits are warmed above the temperature of the air during sunshine, and may attain a temperature of 40° C. (108° F.) when the air is only at a temperature of 28° C. (81° F.). Such high temperatures may possibly explain the speedy ripening of the raspberry.

6. Estimations of the depression of freezing-points of standardized extracts from dormant and from bursting buds of hazel suggest that the sap cannot freeze more easily when the buds begin to open. They had, in fact, a greater depression of freezing-point at this time.

The writer wishes to express his grateful thanks to Dr A. L. Allen, a former collaborator, for much valuable advice and constructive criticism of the physical side of this investigation. Mr J. F. C. Ward of Dalton, Huddersfield, and Mr H. Kitchenman of Waterloo, Huddersfield, have given the use of their orchards, and have afforded much help with the daily records in 1937 and 1938 respectively. Mr C. E. Cornford of East Malling Research Station propounded the questions as to whether the orchard heaters warmed by convection or by direct radiation, and as to possible variations of internal temperatures between adjacent buds. Messrs Geo. Monro, Ltd., of Waltham Cross, have presented four orchard heaters, and Mrs M. Grainger has given continued assistance in the preparation of the manuscript. For all this help, the writer expresses his sincere gratitude.

REFERENCES

CLARK, J. E., MARGARY, I. D. & CAVE, C. J. P. (1933). Report on the phenological observations in the British Isles from December 1931 to November 1932. *Quart. J.R. met. Soc.* **59**, No. 251.

CORNFORD, C. E. (1937). A note on frost damage investigations. *Ann. Rep. E. Malling Res. Sta.* 1936, pp. 126–30.

GRAINGER, J. & ALLEN, A. L. (1936). The internal temperatures of fruit-tree buds. *Ann. appl. Biol.* **23**, 1–10.

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THE MANURIAL REQUIREMENTS OF PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM* TREV.)

By J. T. MARTIN, H. H. MANN and F. TATTERSFIELD

Rothamsted Experimental Station, Harpenden, Herts

(With Plates I and II and 1 Text-figure)

Introduction

It is generally recognized that the insecticidal pyrethrum plant (*Chrysanthemum cinerariaefolium* Trev.) prefers a comparatively dry climate and a well-drained sandy soil, and that its manurial requirements are small.

A valuable account of pyrethrum cultivation in Japan is given in the *Bulletin of the Imperial Institute* (1937). Land exposed to the sun with sandy soil and good drainage is chosen, and stable litter with an auxiliary in the form of night soil, plant ash, fish cake or superphosphate is applied in moderate dressings. In fertile soil, the excessive application of nitrogenous manures results in leaf development at the expense of flower production. The stable litter is used to prepare the land for the seedlings, and the auxiliary manure is applied in the autumn after the flower harvest. Application of the auxiliary manure in the spring results in a reduced yield of flowers.

Gnadinger *et al.* (1936) found that the application of commercial fertilizers had little effect upon the yield of flowers or their content of the pyrethrins. Drain (1936), describing the cultivation of pyrethrum in Tennessee, states that the best results were obtained on fertile sloping soil, and that a moderate application of a complete fertilizer is desirable. Excessive application of nitrogen tended to reduce the yield of flowers, and predisposed the plants to disease. Ripert (1935) recommends the use of a complete fertilizer, stating that in favourable years considerable increases in flower production result, while in unfavourable years a satisfactory yield is maintained. Fertilizers with rapid action are recommended for dry years, and slow-acting fertilizers for wet years. Nitrate fertilizers are not to be recommended. Ripert states, also, that the use of a complete fertilizer has an effect upon the longevity of the crop.

In previous work (Martin & Tattersfield, 1934) experiments were carried out in large pots, using the heavy soil from Broadbalk field,

Rothamsted, which had not been manured for many years and, for each trial, rooted shoots from one parent plant. Weighed quantities of fertilizers were applied at the beginning of each experiment. The results showed that after a slight initial increase in the yield of flowers, the fertilizers had little effect upon the heads produced or upon their content of pyrethrin I. Good yields of flowers rich in pyrethrin I were obtained from the plants grown in the unmanured soil. The pyrethrin I content was found to be more dependent upon a genetical than a nutritional factor. It was hoped to obtain information upon the effect of the manures upon the survival period of the plants, but root development became so excessive as to necessitate the abandonment of the experiments.

The present paper deals mainly with a small-scale field experiment, designed to provide further information on the influence of manures upon the yield of flowers, their content of the pyrethrins, and upon the economic survival period of the plant. The aim of the experiment was discussed and its design decided upon by a committee composed of Mr C. T. Gimingham, Dr D. J. Watson and the authors, the manurial system being finally worked out by Dr D. J. Watson.

Experimental

The experiment was commenced early in 1933 on a hill in Roadpiece field, Woburn, Beds. The experimental area had previously been under grass with lucerne. The soil was very sandy, of the Lower Greensand type, with, in many parts, the characteristic ironstone pieces. The soil overlying the sand was very thin, and in order to bring the area into a low state of fertility and to eliminate any effect due to the previous crop, the surface soil was removed in March 1933 to a depth of 2–3 in. The area was enclosed in pig- and cattle-proof fencing, with wire netting to keep out ground game.

The experimental area was made up of thirty-two plots, each $6\frac{1}{2}$ by 5 yd. or approximately 0·0067 acre in size. Guard rows separated the experimental plants, so that the area of each plot harvested was 0·0056 acre. There were 108 experimental plants in each plot, made up of nine rows of twelve plants each; the rows of plants and the plants in the rows were each 18 in. apart.

The plants used for the experiment were raised from seed at the Plant Pathological Laboratory of the Ministry of Agriculture, and were planted out at the end of May 1933. The lime had been applied to the appropriate plots at the end of April, and the mineral manures were applied immediately before planting. The nitrogenous manures were applied in two dressings, early in June and again in August. The climatic conditions were unsatisfactory for the planting out, and gaps were filled with new seedlings on two occasions in June. By August 1933, the plants were sturdy and were bushing out well, with a few gaps. These were filled in November with plants from the guard rows, and the latter were replanted with new seedlings in May 1934.

Manurial treatments. Two replications of combinations of no lime (O) and lime (L), no fish manure (O) and fish manure (F), no artificials (O) and complete artificials (A),

applied in the first year of the experiment only (1), and applied every year (2), were tested. Lime was applied in the first year of the experiment only (1933) at the rate of 2·88 tons of ground lime, equivalent to 4 tons of calcium carbonate/acre. Where applied in the first year only, fish manure was given at the rate of 5 cwt./acre, equivalent to 0·4 cwt. nitrogen/acre, and where applied every year it was given at half this rate per annum. The complete artificials were applied as sulphate of ammonia, superphosphate and muriate of potash. Where applied in the first year only, they were given at rates equivalent to 0·4 cwt. nitrogen, 0·4 cwt. P_2O_5 and 0·5 cwt. K_2O/acre, and where applied every year half these rates were given per annum. The manures were applied in the spring of each year, those for 1934 being applied in April. The plan of the experiment is given in Text-fig. 1.

1									8
N.W. ↑	LOA 1	LFO 2	OFO 2	LOO 1	OOA 1	LOO 1	OOA 2	OOO 2	
	LFO 1	OOA 2	OOA 1	OFA 2	OFO 1	LOA 2	LOA 1	LFA 1	
	LFA 2	OFO 1	LFA 1	LOA 2	LFO 1	LOO 2	LFO 2	OFA 2	
25	OOO 1	LOO 2	OOO 2	OFA 1	OFA 1	LFA 2	OOO 1	OFO 2	32

O = No application
L = Lime
F = Fish Manure
A = Artificials

1 = Manures applied first year only
2 = Manures applied every year

Lime applied in first year only

Text-fig. 1. Plan of the experimental area.

1934 *harvest*

The crop flowered well in June 1934, and was harvested 4–6 July, when the majority of the flowers were in the fully open condition. Owing to damage to the outside guard rows on the north-west and south-east sides of the experimental area, it was decided to abandon one row at each of these extremities. Eight rows of experimental plants were therefore harvested in plots 1–8 and 25–32. The flowers from each plot were harvested separately. It was found to be most convenient for the flowering stalks of each plant to be loosely tied, the bunches cut by shears, and the heads removed by drawing small bunches through metal combs (see Jary, 1936). The flowers, shielded from the sun by hessian cloths, were dried by exposure in an unheated glasshouse. The stalks were completely

removed, the yield from each plot determined, and representative samples of the air-dried flowers taken for analysis. The pyrethrins I and II were determined by the Tattersfield "acid" method as modified by Martin & Tattersfield (1931). The free acids were not removed and the dicarboxylic acid of the pyrethrin II was extracted with ether in a separating funnel. The yields of air-dried stalkless heads per plot, together with their pyrethrin contents, are given fully in the *Report of the Rothamsted Experimental Station for* 1934 (p. 203). The summarized results of the statistical examination are given later in this paper (Tables I–III).

At the time of the 1934 harvest 8·0% of the experimental plants had failed, and the gaps were filled from the guard rows in November 1934. The area was weeded in October and December 1934, and the manures were again applied, where required, in March 1935.

1935 *harvest*

The plants began to flower vigorously at the end of May, and all nine rows of each plot were harvested 8–12 June. Photographs were taken immediately before harvest (Pl. I, figs. 1, 2). The heavy crop necessitated five people beheading at the combs for the greater part of the $4\frac{1}{2}$ days required for the harvesting. The flowers from each plot were dried as before, the stalks fully removed from a representative sample, and the weight of air-dried stalkless heads per plot calculated. The representative samples were analysed for pyrethrins I and II by the method of Seil (1934). The yields of air-dried stalkless flowers per plot are given in the *Report of the Rothamsted Experimental Station for* 1935 (p. 201), but the pyrethrin contents are not included, as they were determined at a later date. The values obtained have, however, been incorporated for reference purposes in the *Report of the Rothamsted Experimental Station for* 1937. The summarized results, following statistical examination, are given in Tables I–III.

The extent of failure of the experimental plants at the time of the 1935 harvest amounted to 5·7%. Large differences were seen in the development of weeds on the plots, those receiving fish manure or fish manures and artificials every year being generally in a worse condition than the plots receiving manures in the first year only. The plots were cleaned in September 1935. The gaps were not filled and the manures were again applied, where necessary, in April 1936.

1936 *harvest*

The flowers were harvested 7–10 July. Owing to the failure of plants in the outside guard rows of plots 1–8 and 25–32, only eight rows in these plots were harvested as in 1934. The flowers from each plot were weighed in the fresh condition, and a representative sample taken, on which the yield of oven-dried (100° C.) stalkless flowers was determined. The yields of dry stalkless heads were then calculated for the plots. The pyrethrins I and II contents were determined on air-dried flowers by the method of Seil, with preliminary removal of the free acids from the petroleum-ether extracts. The yields of oven-dried stalkless flowers per plot, with the pyrethrin contents of the air-dried flowers, may be obtained by reference to the *Report of the Rothamsted Experimental Station for* 1936 (p. 231). The summarized results, after statistical examination, are included in Tables I–III.

Records were taken of the numbers of plants surviving at the time of the 1936 harvest. Of the experimental plants, 20·1% had failed. The plants in plots 1–8 were smaller than the remainder, and in these plots 32·4% of the plants had died. Of the rows of plots, the second row made up of plots 9–16 showed 19·9% of plants missing, while plots 17–24 showed 14·0%, and plots 25–32 14·1% of failures. The progressive decrease in the percentage death of plants from plots 1–8 to plots 17–24 was probably influenced largely by the fact that the experimental area was not on level ground. Plots 1–8 were on the crest of the hill facing the north-west, and had experienced the full force of the winds from the north, while plots 9–16 and 17–24 were increasingly sheltered by the brow of the hill and by the plants in plots 1–8.

The experimental area was hand-hoed in August 1936, and again completely cleaned prior to the application of the manures in April 1937. It was decided at this stage to omit plots 1–8 and 25–32 when taking the 1937 harvest owing to the failure of the plants in plots 1–8 and the loss of the guard row in plots 25–32. The plots receiving manures in the first year only were to be regarded in the subsequent statistical analysis as unmanured plots, there being little chance of any residual manurial effect.

1937 *harvest*

The flowers from plots 9–24 were taken on 28 and 29 June. The fresh flowers from each plot were weighed, and the dry matter (100° C.) and stalk content determined on representative samples. The pyrethrins I and II were determined on air-dried stalkless flowers, containing approxi-

mately 12% of moisture, by the method of Seil, incorporating the preliminary removal of the free acids. The yields of oven-dried flowers for the plots harvested may be obtained from the *Report of the Rothamsted Experimental Station for* 1937, while the summarized results of the statistical examination of the data are included in Tables I–III.

Table I. *The effect of lime, applied in the first year only, upon the yield of flowers and their content of the pyrethrins*

Year	No lime	Lime	Difference	S.E. of diff.
		*Dry flowers (cwt./acre)		
1934	4·70	5·14	+0·44	±0·29
1935	6·72	7·32	+0·60	±0·37
1936	4·93	5·28	+0·35	±0·33
1937	4·26	4·86	+0·60	±0·45
		Pyrethrin I (% of flowers)		
1934	0·528	0·559	+0·031	±0·0201
1935	0·449	0·472	+0·023	±0·0207
1936	0·406	0·420	+0·014	±0·0231
1937	0·520	0·545	+0·025	±0·0183
		Total pyrethrins (% of flowers)		
1934	1·141	1·196	+0·055	—
1935	1·006	1·051	+0·045	—
1936	0·868	0·931	+0·063	—
1937	1·230	1·250	+0·020	—

Table II. *The effect of manures, applied in the first year only, upon the yield of flowers and their content of the pyrethrins*

Year	No manures	Artificials	Fish manure	Artificials plus fish manure	S.E.	Mean of manures
			*Dry flowers (cwt./acre)			
1934	4·94†	4·16	4·82	3·58	±0·404	4·19
1935	6·62†	7·00	7·75	7·51	±0·520	7·42
1936	5·27†	5·28	5·12	5·38	‡	5·26
1937	—	—	—	—	—	—
			Pyrethrin I (% of flowers)			
1934	0·55†	0·55	0·52	0·55	±0·0284	0·54
1935	0·46†	0·50	0·44	0·46	±0·0246	0·47
1936	0·38†	0·39	0·40	0·37	±0·0327	0·39
1937	—	—	—	—	—	—
			Total pyrethrins (% of flowers)			
1934	1·18	1·16	1·15	1·24	—	1·18
1935	1·01	1·11	1·00	1·03	—	1·05
1936	0·86	0·88	0·86	0·82	—	0·85
1937	—	—	—	—	—	—

* Yields for 1934–5 are on air-dry basis; for 1936–7 on oven-dry basis.

† The standard errors of these figures are $1/\sqrt{2}$ times the standard errors given for the corresponding manured plots.

‡ No single standerd error is applicable to these figures.

Table III. *The effect of manures, half quantities applied every year, upon the yield of flowers and their content of the pyrethrins*

Year	No manures	Artificials	Fish manure	Artificials plus fish manure	S.E.	Mean of manures
			*Dry flowers (cwt./acre)			
1934	4·94†	5·28	6·03	5·60	±0·404	5·64
1935	6·62†	6·84	6·38	7·42	±0·520	6·88
1936	5·27†	5·00	4·76	4·78	‡	4·85
1937	4·17†	4·58	5·25	5·49	‡	5·11
			Pyrethrin I (% of flowers)			
1934	0·55†	0·54	0·54	0·54	±0·0284	0·54
1935	0·46†	0·45	0·45	0·46	±0·0246	0·45
1936	0·38†	0·47	0·46	0·45	±0·0327	0·46
1937	0·51†	0·57	0·55	0·55	‡	0·56
			Total pyrethrins (% of flowers)			
1934	1·18	1·14	1·14	1·16	—	1·15
1935	1·01	1·02	1·04	1·02	—	1·03
1936	0·86	1·00	0·95	0·97	—	0·97
1937	1·22	1·27	1·24	1·27	—	1·25

* Yields for 1934–5 are on air-dry basis; for 1936–7 on oven-dry basis.

† The standard errors of these figures are $1/\sqrt{2}$ times the standard errors given for the corresponding manured plots.

‡ No single standard error is applicable to these figures.

In the plots harvested, 28·1% of the plants had failed, there being a greater loss in plots 9–16 (32·8%) than in plots 17–24 (23·5%). After the 1937 harvest the roots of one of the experimental plants were excavated to determine the extent of root development. There was comparatively little lateral spread, while from the mass of root material clustered near the surface, intertwined fine roots penetrated the sand to a depth of 5 ft. (Pl. II).

Weed population

The experimental area was not cultivated after August 1937, and observations on the prevalence of weeds were made in April 1938. Of the annual weeds, no chickweed, spurry or mayweed, and very little *Veronica*, were found, although these were abundant in the surrounding fields. *Poa annua* was abundant, and groundsel, *Alchemilla* and small leguminous weeds, probably wild clovers, were present. There had been a considerable amount of *Polygonum aviculare* in 1937, but no new seedlings were visible. There were many more perennial weeds, and these, particularly the grasses, were very abundant. Grasses predominated, chiefly *Agrostis stolonifera* and *Festuca ovina*, both very common in the neighbourhood. There were also many thistles, and patches of *Convolvulus*. There were at this stage no apparent differences in weed development between the plots manured in various ways or due to the effect of lime.

Climatic conditions

The months of 1933 during which the seedlings were establishing themselves were abnormally sunny, warm and dry, the succeeding winter months being normal. 1934 was generally a year of average sunshine and temperature, with low rainfall. July was sunnier and warmer than average, while December was particularly warm and wet. 1935 showed in general normal sunshine and temperatures, July again being sunnier, warmer and drier than usual. April, September and particularly November were wet months. The year 1935 was characterized by severe frost conditions in May, a minimum grass temperature of 21·5° F. being recorded on 17 May. The year 1936, and the first six months of 1937 were periods of reduced sunshine, generally normal temperatures, and abnormally high rainfall.

Discussion

The outstanding result has, perhaps, been the high yields of flowers obtained throughout the experiment, in view of the infertile nature of the soil used. The yield reached a maximum in 1935, two years after the seedlings were planted out, in spite of the severe frosts in May which almost completely checked the flowering of other experimental beds of pyrethrum in lower-lying localities. Lime, which was applied in the first year only, increased the yield of flowers slightly each year (Table I). The increases were not individually significant in any year, and their regularity may partly be due to the fact that the same plots persisted throughout the experiment. The effect of lime upon the pyrethrin I contents of the flowers was very small but positive. The results are in agreement with the view generally held that lime is beneficial to the production of flowers.

Following the liberal application of manures in the first year only, a significant depression in the yield of flowers resulted in the following year (1934), succeeded by an almost significant increase in 1935, and no effect in 1936 (see Table II). There was no effect upon the pyrethrin I or total pyrethrin contents of the flowers. The initial depression in the yield of flowers obtained in 1934 may have resulted from too great a vegetative development of the plants, at the expense of flower production. This effect has been noted before, when plants showing a large, bushy development have produced few flowers.

When the manures were applied in moderate dressings every year, a significant increase in the yield of flowers resulted in 1934 and again in 1937 (see Table III). In 1935 the application of manures had no effect,

and the apparent depression in 1936 did not approach significance. In the two years in which the manures increased the yield of flowers, fish manure gave somewhat higher yields than did the artificials, though the differences were not significant. Manures applied every year had no effect upon the pyrethrin I content of the flowers in 1934 and 1935, but produced significant increases in 1936 and 1937. We have grown pyrethrum experimentally at various centres in England, and have noticed that, in general, the maximum yield of flowers is obtained in the second year after planting out, followed by a gradual drop in the total pyrethrin content of the flowers. The yearly application of moderate dressings of manures in this experiment has had the apparent effect of delaying this fall in pyrethrin content in the fourth and fifth years of the experiment. In all cases, the effects on the total pyrethrins were similar to those on the pyrethrin I content of the flowers.

The pyrethrin contents of the flowers in 1936 were generally lower than in the previous years, particularly on the unmanured plots, and this fact may have been connected with the adverse climatic conditions prevailing during the first half of the year. March, April, May and June were overcast months, while June was particularly wet. The first six months of 1937, however, were all dull and wet, and yet the pyrethrin contents of the flowers were amongst the highest recorded during the experiment. The high values for the 1937 flowers may have been influenced by the low pyrethrin contents of the flowers of the previous year.

There was no evidence in the fifth year of the experiment that the yearly application of moderate dressings of manures had played any part in prolonging the survival period of the crop, judged from the percentages of plants surviving. The plants grown in the plots to which no manures had been supplied were vigorous in the fifth year of the experiment, showed no greater percentage of failure than the plants grown in the manured plots, and flowered at the rate of 4 cwt. of dry flowers/acre. There was, however, some indication that lime produced a slight increase in plant survival in the last two years, as the following figures for the percentages of plant failure show:

	1934	1935	1936	1937
No lime	8·1	5·5	23·3	31·2
Lime	7·9	5·7	16·9	25·1

The manurial requirements of pyrethrum would appear to be surprisingly small, but the possible beneficial effect of moderate yearly dressings of manures upon the yield of flowers and upon the pyrethrin

content is seen, particularly in the last year of the experiment. The application of the manures in the autumn after flowering may prove advantageous, but was not tested in the present experiment as it was felt that manures so applied would rapidly be lost from the sandy soil by winter rain. The use of dung and organic manures may also have a valuable effect in improving the tilth of the soil. Further experimentation is required and should take place in the localities in which pyrethrum is grown commercially.

A note by Mr W. G. Cochran upon the statistical examination of the data is appended.

Statistical note. The experiment consisted of two randomized blocks of 16 treatments each and the statistical analysis followed the usual method for randomized blocks. In 1937 only half of the experiment was harvested and as this did not coincide with a block, an analysis was performed by the method of least squares, ignoring the difference between blocks. The standard errors per cent per plot may be of interest to those contemplating experiments on this crop. For dry flowers, they were 16·4% in 1934, 14·8% in 1935, 13·0% in 1936 and 17·8% in 1937. These figures are considerably higher than the usual average of 8–12% for English farm crops. It should be noted, however, that the plot size was only 0·0056 acre and that the positions of the blocks turned out to be unfortunate. Had the comparative failure of the outside rows (north-west and south-east) been foreseen at the start, a design in four randomized blocks of eight plots would have been more accurate. Further, owing to the factorial design used, the replication on the effect of lime was sixteenfold.

The pyrethrin figures in Table I will be found to differ slightly from the corresponding figures in the Rothamsted Experimental Station Report owing to the use of an extra decimal place in the calculations in this paper.

Summary

A small field experiment upon the manurial requirements of the insecticidal pyrethrum plant, grown upon sandy soil of low fertility, is described. Lime had the effect of producing slight, but not significant, increases each year in the yield of flowers and their content of the pyrethrins, and decreased the percentages of plant failures in the fourth and fifth years of the experiment. There was a significant depression in the yield of flowers in the year after the single application of double dressings of the manures, but no effect in later years. The yearly application of moderate dressings of manures gave significant increases in the

yield of flowers in the second and fifth years, and significant increases in the pyrethrin I content of the flowers in the fourth and fifth years of the experiment.

We wish to convey our thanks to Mr C. T. Gimingham for raising the seedlings used in the experiment and for helpful criticism and advice, to Dr D. J. Watson for assistance in drawing up the plan of the experiment, to the staff of the Woburn Experimental Station for assistance in the field work, and to Mr W. G. Cochran for carrying out the statistical examinations. The photographs were taken by Mr V. Stansfield.

REFERENCES

Anon. (1937). The cultivation of pyrethrum in Japan. *Bull. Imp. Inst., Lond.*, **35**, 318.

Drain, B. D. (1936). Pyrethrum in Tennessee. *Circ. Tenn. agric. Exp. Sta.* no. 59.

Gnadinger, C. B., Evans, L. E. & Corl, C. S. (1936). Pyrethrum plant investigations in Colorado. *Bull. Colo. agric. Exp. Sta.* 428.

Jary, S. G. (1936). Pyrethrum. *J. S.-E. agric. Coll. Wye*, no. 38, p. 59.

Martin, J. T. & Tattersfield, F. (1931). The evaluation of pyrethrum flowers (*C. cinerariaefolium*). *J. agric. Sci.* **21**, 115.

—— —— (1934). The effect of environmental conditions upon pyrethrum (*C. cinerariaefolium*). I. *Ann. appl. Biol.* **21**, 670.

Rep. Rothamst. exp. Sta. (1934–7).

Ripert, J. (1935). *Le pyrèthre français.* Paris: Dehon and Co.

Seil, H. A. (1934). Estimation of pyrethrins. *Soap*, **10**, No. 5, 89.

EXPLANATION OF PLATES I AND II

PLATE I

Fig. 1. The experimental plot immediately before the 1935 harvest.

Fig. 2. Near view of the experimental plot immediately before the 1935 harvest.

PLATE II

The excavated root system of a single plant of *Chrysanthemum cinerariaefolium*.

(*Received* 16 *June* 1938)

Fig. 1.

Fig. 2.

MARTIN, MANN AND TATTERSFIELD.—THE MANURIAL REQUIREMENTS OF PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM* TREV.) (pp. 14–24)

MARTIN, MANN AND TATTERSFIELD.—THE MANURIAL REQUIREMENTS OF PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM* TREV.) (pp. 14–24)

THE ECOLOGY OF THE LARGER FUNGI

III. CONSTANCY AND FREQUENCY OF GRASSLAND SPECIES WITH SPECIAL REFERENCE TO SOIL TYPES

By W. H. WILKINS and SHEILA H. M. PATRICK

Mycology Laboratory, University Department of Botany, Oxford

CONTENTS

I. Introduction

The present work was carried out by the junior author:[1] the senior author is responsible for suggesting the problem, supervising the work and preparing the paper for the Press. The work is a continuation of the investigation into the ecological distribution of the larger fungi (Wilkins *et al.* 1937).

II. Previous literature

Literature in connexion with the ecology of fungi in general has been dealt with (Wilkins *et al.* 1937). Literature on grassland fungi is even less adequate than that on woodland species. Graham (1927), in discussing the relative abundance of woodland and grassland species at different

[1] In partial requirements for the B.Sc. degree.

seasons of the year in the region round Chicago, says that in autumn fungi are absent from the pastures due to drought but present in woods owing to the moister conditions prevailing there. In spring, on the other hand, the forest canopy retards the rise of temperature and fungi are scarce in woods but plentiful in pastures due to the spring rains and the more rapid rise of temperature on the exposed ground.

Gilbert (1928) deals in a general way with the climatic conditions influencing fungus production in both woodlands and grasslands, and discusses the effect of various edaphic factors on fungus frequency.

III. Experimental method

Twenty different grassland stations, chosen within a 15 mile radius of Oxford, Leicester, and Haslemere (Surrey) respectively, were examined. These twenty stations comprised three soil types, chalk, clay and sand, two stations on limestone being included under the heading of chalk. This arrangement postulated that any variation in different soil types was a general factor and was not specifically influenced by local conditions. Table I shows the district and soil type of each of the stations, with the names by which they will subsequently be known.

Table I. *List of the twenty stations examined*

	Chalk	Clay	Sand
Haslemere	Graffham Down Heyshott Down	Frillinghurst Meadows Gospel Green	North Heath Valewood Meadows
Leicester	Waltham Meadows	Evington Meadows Langton Meadows	Bradgate Park Donington Meadows Rothley Meadows
Oxford	Blenheim Park Chinnor Hill Crowell Hill White Horse Hill	Horspath Meadows Waterperry Common	Nuneham Park Turville Heath

Table I shows that seven stations are on chalk, six on clay and seven on sand. Owing to the geological conformation of the districts it was not possible to get an equal number of stations of each soil type in each district, e.g. chalk was represented by only one station in the Leicester district but by four stations in the Oxford district.

Stations were selected as being homogeneous as far as could superficially be determined. In each station holes were dug and soil profiles and textures were examined. The organic content of the upper 2 in. of the soil was estimated by the method of Walkley & Black (1934) and is recorded for each station as an average of ten determinations. The *p*H values of the top 1 in. of soil were determined by the B.D.H. Capillator and, variations of different determinations on any one station being insignificant, the average value is given for each station. Notes of the floristic composition of the stations were made in detail as being valuable indications of the soil type.

Lists of the fungi found on all stations were made once a fortnight in the autumns of 1936 and 1937. The method of listing was to traverse the station backwards and forwards a number of times on the "zigzag transect" principle. The areas were not measured, but some approximation to equality of area in the different stations was

ensured by carrying on each examination for 3 hr. Detailed estimation of frequency was impossible, but reasonable accuracy was attained by experience. At each visit the number of sporophores of each species was estimated approximately and recorded by a symbol indicating frequency, using the following scheme, approximately 1 sporophore = *R*, ±10 = *O*, ±100 = *F* and ±500 = *A*. This, with allowance for intermediates, enabled frequency to be expressed adequately within somewhat wide limits, e.g. 1000–1500 individuals, rather than by actual numerical values.

IV. Ecological notes on the twenty stations

Notes of the principal phanerogams were taken during May and June 1937. The plants were named according to Hooker (1930) and listed with an approximate estimation of frequency. These notes, together with the general characters of the soil, are given below for each station. The stations are classified into three groups corresponding to the three soil types, and are arranged in order of increasing acidity.

Section I. Chalk

(1) *White Horse Hill.* Chalk. *p*H 7·2.

Short, sheep-grazed turf on plateau. Black, crumbly, humus-stained loam 3–9 in. Dark grey loams more compact with occasional chalk fragments 6–18 in. Solid chalk in some places below 10 in., in others considerably deeper. Organic content 21·1%.

Bromus erectus and *Festuca ovina* l.d., *Deschampsia caespitosa, Bellis perennis, Helianthemum vulgare* and *Poterium Sanguisorba* a., *Brachypodium pinnatum, Briza media, Cnicus acaulis, Hieracium Pilosella, Lotus corniculatus, Plantago lanceolata* and *Ranunculus bulbosus* f., *Anthyllis Vulneraria, Dactylis glomerata, Rumex Acetosella* and *Thymus Serpyllum* o.

(2) *Waltham Meadows.* Lincolnshire limestone. *p*H 7·2.

Light, friable, brown loam varying in depth 4–12 in. Rapid increase of flints and limestone fragments below this, with solid limestone varying in depth 2–5 in. Organic content 12·8%. Occasional *Crataegus Oxyacantha.*

Festuca ovina and *Poa pratensis* a., *Arrhenatherum avenaceum, Briza media, Dactylis glomerata, Hieracium Pilosella, Holcus lanatus, Leontodon hispidus, Medicago lupulina, Plantago lanceolata, Polygala vulgaris, Poterium Sanguisorba* and *Ranunculus bulbosus* f., *Brachypodium pinnatum, Carex glauca, Carlina vulgaris, Cirsium acaulis* and *Lotus corniculatus* o., *Ophrys apifera* r.

(3) *Blenheim Park.* Cornbrash. *p*H 7·1.

Friable, black-brown, humus-stained loam 3–6 in. Greyish brown with slight humus staining, more compact, with frequent small flints and limestone particles 10–14 in. Becoming lighter in colour with frequent large limestone particles 18–24 in. Solid limestone below this. Some mole burrowing. Organic content 16·0%.

Arrhenatherum avenaceum, Brachypodium pinnatum, Festuca ovina and *Hypnum cupressiforme* l.d., *Anthoxanthum odoratum, Cynosurus cristatus, Nepeta Glechoma, Poa pratensis, Senecio Jacobaea* and *Veronica Chamaedrys* f., *Helianthemum vulgare, Poterium Sanguisorba, Poa trivialis* and *Viola hirta* o.

(4) *Chinnor Hill.* Chalk. *p*H 7·0.

Escarpment slope facing south-west. Short, spongy, rabbit-grazed turf. Black, crumbly loam with occasional small chalk fragments 5–6 in. Grey loam with larger chalk particles 9–15 in. Solid chalk below 9–15 in. Organic content 20·7%. Scattered *Juniperus communis, Ligustrum vulgare, Rosa* spp. and *Rubus* spp.

Festuca ovina d., *Brachypodium pinnatum* l.d., *Poterium Sanguisorba* a., *Anthoxanthum odoratum, Bellis perennis, Carex glauca, Helianthemum vulgare, Leontodon hirtus* and *Plantago media* f., *Briza media, Campanula glomerata, Carlina vulgaris, Cnicus acaulis, Galium verum, Polygala vulgaris, Plantago lanceolata, Primula veris* and *Thymus Serpyllum* o.

(5) *Crowell Hill.* Chalk. *p*H 6·9.

Escarpment slope similar to Chinnor. Black, crumbly, humus-stained loam 3–4 in. Grey-black loam with occasional small chalk fragments 7–8 in. Light grey loam, sticky and more compact, with abundant chalk fragments 10–18 in. Solid chalk below 14–20 in. Organic content 25·7%. *Crataegus Oxyacantha* o., and *Rosa* spp. r.

Brachypodium pinnatum, Briza media, Carex glauca, Festuca ovina and *Hypnum cupressiforme* a., *Dactylis glomerata, Galium verum, Hieracium Pilosella, Leontodon hirtus* and *Poterium Sanguisorba* f., *Campanula glomerata, Cnicus eriophorus* and *Viola hirta* o.

(6) *Graffham Down.* Chalk. *p*H 6·75.

Down on ridge of escarpment, extending east to west. Approximately level. Sheep and rabbit-grazed turf, compact and spongy. Black, crumbly, humus-stained loam 3–4 in. Dark grey loam becoming lighter in colour with rapid increase of chalk fragments down to 18 in., as far as investigated. A considerable clay fraction was present in some parts. Organic content 30·2%. *Crataegus Oxyacantha, Fraxinus excelsior* and *Rubus* spp. were rarely present.

Festuca ovina, Hypnum Schreberi and *Poterium Sanguisorba* a., *Briza media, Carex glauca, Cnicus acaulis, Galium verum, Hieracium Pilosella, Hypnum cupressiforme, Brachypodium pinnatum* and *Helianthemum vulgare* f.

(7) *Heyshott Down.* Chalk. *p*H 6·7.

Similar to Graffham, on same escarpment ridge. Short, compact, spongy turf, much mole burrowing. Black, crumbly loam with occasional small chalk fragments 4–6 in. Dark grey loam with increase of flints and chalk fragments becoming more compact to 18 in., as far as investigated. Organic content 27·0%. *Crataegus Oxyacantha* and *Rubus* spp. occasional.

Carex glauca, Festuca ovina, Hypnum Schreberi and *Poterium Sanguisorba* a., *Briza media, Cnicus acaulis, Galium verum, Hieracium Pilosella* and *Viola hirta* f.

Section II. Clay

(8) *Langton Meadows.* Boulder Clay. *p*H 6·2.

Heavy clay loam with slight humus-staining 3–4 in., becoming paler in colour with increasing clay fraction, gley below 8–10 in. Organic content 11·9%.

Alopecurus pratensis, Anthoxanthum odoratum, Cynosurus cristatus, Poa trivialis, Ranunculus acris and *Trifolium repens* a., *Bellis perennis, Cerastium vulgare, Cnicus lanceolatus, Dactylis glomerata, Rumex Acetosa* and *Trifolium pratense* f.

(9) *Evington Meadows.* Upper Lias Clay. *p*H 6·05.

Heavy, dark brown clay loam, humus-stained 2–3 in. Paler in colour with increase of clay fraction and some gley to 18 in., as far as investigated. Organic content 12·5%.

Bellis perennis, Cynosurus cristatus, Poa pratensis, P. trivialis, Ranunculus acris and *R. bulbosus* a., *Alopecurus pratensis, Anthoxanthum odoratum, Centaurea nigra, Cnicus lanceolatus, Dactylis glomerata, Holcus lanatus, Lolium perenne* and *Trifolium pratense* f., *Conopodium denudatum, Galium verum, Plantago lanceolata, Poterium officinale, Rhinanthus Crista-galli* and *Rumex Acetosella* o.

(10) *Horspath Meadows.* Kimeridge Clay. *p*H 6·15.

Heavy, dark brown clay loam, humus-stained 2–3 in. Becoming lighter in colour with gley formation below 6–8 in. Organic content 9·4%.

Cynosurus cristatus, Dactylis glomerata, Plantago lanceolata and *Poa pratensis* a., *Bellis perennis, Leontodon hirtus, Ranunculus acris, R. bulbosus, Trifolium pratense* and *T. repens* f., *Rhinanthus Crista-galli* and *Rumex Acetosa* o.

(11) *Waterperry Common.* Oxford Clay. *p*H 6·0.

Heavy, dark brown clay loam, humus-stained 2–3 in. Becoming rapidly paler and greyer in colour with gley formation below 12–18 in. Organic content 11·2%.

Bellis perennis, Cynosurus cristatus, Poa pratensis and *Ranunculus acris* a., *Carex panicea, Deschampsia caespitosa, Festuca ovina, Holcus lanatus, Prunella vulgaris, Trifolium pratense, T. procumbens* and *T. repens* f., *Cerastium vulgare, Chrysanthemum Leucanthemum, Lolium perenne, Luzula pratensis* and *Ranunculus repens* o.

(12) *Frillinghurst Meadows.* Wealden Clay. *p*H 6·0.

Compact, heavy, brown clay loam, humus-stained to 4 in. Then clay fraction predominating with gley formation below 7 in. Organic content 8·5%.

Anthoxanthum odoratum, Dactylis glomerata, Plantago lanceolata and *Ranunculus acris* a., *Alopecurus pratensis, Bellis perennis, Centaurea nigra, Chrysanthemum Leucanthemum, Lolium perenne, Rhinanthus Crista-galli, Rumex Acetosa, R. Acetosella* and *Trifolium pratense* f.

(13) *Gospel Green.* Wealden Clay. *p*H 5·85.

Compact, heavy, brown loam, humus-stained 1–2 in. Becoming greyer in colour with predominating clay fraction, gley below 9 in. Organic content 13·3%.

Anthoxanthum odoratum, Bellis perennis, Plantago lanceolata and *Ranunculus acris* a., *Alopecurus pratensis, Dactylis glomerata, Centaurea nigra, Lolium perenne, Rumex Acetosa* and *Veronica Chamaedrys* f.

Section III. Sand

(14) *Donington Meadows.* Marl and sandstone. *p*H 5·9.

Light, red-brown, sandy loam, slight humus-staining to 3 in. Becoming paler in colour but uniform in texture to 18 in., as far as investigated. Organic content 9·6%.

Anthoxanthum odoratum, Cynosurus cristatus, Festuca ovina and *Ranunculus acris* a., *Centaurea nigra, Cnicus lanceolatus, Holcus lanatus* and *Luzula campestris* f., *Dactylis glomerata, Lolium perenne, Plantago lanceolata* and *Rumex Acetosella* o.

(15) *Rothley Meadows.* Sand and gravel. *p*H 5·75.

Red-brown, friable loam, with humus-staining 2–3 in., then more or less uniform to 24 in., as far as investigated. Small stones below 12 in. Organic content 7·4%.

Holcus lanatus, Poa trivialis and *Ranunculus acris* a., *Anthoxanthum odoratum, Bellis perennis, Festuca ovina, Leontodon hispidus, Plantago lanceolata, Poa pratensis* and *Trifolium pratense* f., *Alopecurus pratensis, Cerastium vulgare, Cynosurus cristatus, Dactylis glomerata, Lolium perenne* and *Rumex Acetosella* o.

(16) *Nuneham Park.* Lower Greensand. *p*H 5·1.

Dark, red-brown, sandy loam, humus-stained to 3 in. Light brown sand, more compact, to 18 in., slight traces of leaching and some iron accumulation at 18–20 in. Considerable clay fraction present in some places, giving a moderately heavy brown loam, very uniform in texture to 9 in., then becoming more compact and iron-stained to 18 in., as far as examined. Organic content 11·3%.

Festuca ovina l.d., *Deschampsia flexuosa, Galium verum* and *Rumex Acetosella* a., *Cnicus arvensis* l.a., *Anthoxanthum odoratum, Hypnum cupressiforme, Luzula campestris, Potentilla erecta, Poa pratensis, Ranunculus acris, Rumex Acetosa, Stellaria graminea* and *Trifolium repens* f., *Cynosurus cristatus, Dactylis glomerata, Hieracium Pilosella, Holcus lanatus, Poa pratensis* and *Veronica montana* o.

(17) *North Heath.* Lower Greensand. *p*H 5·1.

Short, compact turf. Soil very uniform, black, humus-stained sand to 9 in., becoming paler in colour with some iron accumulation below 16 in. Stones below 12 in. Organic content 5·4%.

Anthoxanthum odoratum, Festuca ovina and *Luzula campestris* a., *Bellis perennis, Dactylis glomerata, Holcus lanatus, Ranunculus bulbosus, Rumex Acetosa* and *R. Acetosella* f.

(18) *Bradgate Park.* Marl and sandstone. *p*H 4·95.

Grass heath consisting of short, compact turf, sheep- and rabbit-grazed. Soil very shallow, with frequent rock outcrops. Immature podsol. Grey-black, humus-stained, sandy loam 2–3 in. Then becoming grey-brown and lighter in colour, partly leached in some places, to 8–10 in. Rapid increase of rock fragments with some iron accumulation. Solid rock at 18 in. in some places, soil deeper elsewhere. Organic content 10·4%.

Festuca ovina d., *Anthoxanthum odoratum, Bellis perennis* and *Luzula campestris* a., *Cerastium vulgare, Cynosurus cristatus, Holcus lanatus, Leontodon hispidus, Plantago lanceolata, Ranunculus acris* and *Veronica montana* f., *Galium saxatile, Hieracium Pilosella, Poa pratensis, Potentilla erecta, Ranunculus bulbosus* and *Veronica Chamaedrys* o.

(19) *Valewood Meadows.* Lower Greensand. *p*H 4·95.

Short, compact, grazed turf. Soil varying from a light sandy loam, very loose in texture with frequent stones and traces of iron accumulation about 18 in., to a more definitely podsolized type, with dark, humus-stained sand to 6 in., light grey sand 12 in., whitish leached sand 20 in., and pan formation about 26 in. Organic content 7·6%.

Festuca ovina d., *Cynosurus cristatus, Holcus lanatus, Leontodon hirtus, Luzula pratensis* and *Potentilla erecta* a., *Anthoxanthum odoratum, Dactylis glomerata, Galium saxatile, G. verum, Plantago lanceolata, Poa trivialis, Rumex Acetosella* and *Veronica Chamaedrys* f.

(20) *Turville Heath.* Sand deposit on chalk plateau. *p*H 4·75.

Grass heath consisting of compact, short, spongy turf, sheep- and rabbit-grazed. Typical podsolized type, crumbly, black, humus-stained sand 2 in. Lighter grey-brown sand with flints 10 in. Pale grey, leached sand to about 16 in. Humus accumulation and pan formation 20–24 in. Organic content 14·4%. *Pteridium* and *Ulex europaeus* l.d., *Rubus* spp. o., *Betula pubescens* and *Quercus* r.

Festuca ovina l.d.-a., *Galium verum* l.d., *Luzula pratensis* and *Rumex Acetosella* a., *Potentilla erecta, Poa pratensis* and *Trifolium repens* f., *Anthoxanthum odoratum, Cynosurus cristatus, Hieracium Pilosella* and *Holcus lanatus* o.

V. The fungi—general observations

The total number of species found on all the stations was 147 in 1936 and 125 in 1937. Of these, 100 species were common to the two years, so that the total number of species listed was 172. This indicates a considerable similarity of fungus flora which is, probably, due to the fact that somewhat similar climatic conditions obtained during the two seasons. Both were considered as unfavourable for fungi, being rather cold and dry, and this was more particularly the case in 1937, in which year both constancy of species and frequency of individuals were lower than in 1936.

Certain species, though growing in the grass, were never more than a few yards from a tree, and as these were recognized as typically woodland species they are excluded from the list of grassland fungi. Table II gives these species together with the name of the nearest tree.

Certain other species were not regarded as true grassland fungi in that they were invariably found on tree stumps or rotten wood. These are given in Table III.

(*a*) *Constancy of species*

The general constancy of the 172 species found on the twenty stations in the two years is given in Table IV. As in the first paper (Wilkins *et al.* 1937), the species which were present to a degree of constancy below 20%, i.e. those found in only four out of the twenty stations, are regarded as sporadics, though in this case the sporadic nature of some of these species is largely accounted for by their restriction to one or other of the soil types.

Table II. *Species excluded as being woodland fungi*

Species	Nearest tree
Amanita muscaria	*Betula*
A. rubescens	*Quercus*
Amanitopsis strangulata	,,
A. vaginata	,,
Boletus chrysenteron	,,
B. elegans	*Pinus*
B. erythropus	*Quercus*
B. luteus	*Pinus*
B. satanus	*Fagus*
B. scaber	,,
B. subtomentosus	,,
B. versipellis	,,
Hygrophorus eburneus	,,
Laccaria laccata	*Quercus*
L. laccata var. *amethystina*	,,
Lactarius blennius	*Fagus*
L. insulsus	*Betula*
L. pallidus	*Quercus*
L. pyrogalus	*Betula*
L. quietus	*Quercus*
L. theiogalus	*Pinus*
L. turpis	*Fagus*
Russula atropurpurea	,,
R. cutifracta	,,
R. cyanoxantha	,,
R. drimeia	*Pinus*
R. emetica	*Fagus*
R. fellea	,,
R. foetens	,,
R. fragilis	*Quercus.*
R. heterophylla	*Betula*
R. lepida	*Quercus*

Table III. *Species excluded as being saprophytic on wood*

Armillaria mellea	*Mycena galericulata*
Auricularia mesenterica	*M. polygramma*
Collybia radicata	*Pholiota radicosa*
Coprinus micaceus	*P. squarrosa*
Flammula carbonaria	*Pleurotus applicatus*
F. ochroleuca	*Pluteus cervinus*
Hypholoma fasciculare	*Polyporus brumalis*
H. hydrophilum	*Polystictus versicolor*
H. velutinum	*Tricholoma rutilans*
Lycoperdon pyriforme	*Xylaria hypoxylon*

Table IV. *Constancy of species in the twenty stations*

20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
1	0	3	4	1	4	4	3	6	7	1	3	3	9	9	13	10	26	14	51
															71				101

It is interesting to compare the ratio of constants to sporadics, as illustrated in Table IV, with the corresponding ratios as determined respectively for the twenty oakwoods and the twenty beechwoods, as given by Wilkins *et al.* (1937).

Table V. *Comparison of constancy in grasslands, oakwoods and beechwoods*

	% constant	% sporadic
Grasslands	40	60
Oakwoods	30	70
Beechwoods	26	74

Table V shows that the general relation between constants and sporadics is reasonably consistent, but that on the whole the mycological flora of a given number of grassland stations gives a slightly higher degree of constancy than do the floras of the same number of stations in oakwoods or in beechwoods. In this connexion, however, it must be realized that the two cases are not strictly comparable, as in the case of the grasslands the twenty stations were each examined twice, while in the case of the woodlands the twenty stations comprised some which were examined once and some which were examined several times.

In neither oak nor beech was there any species which occurred with 100% constancy, and in grasslands the only one which was found in all stations was *Hygrophorus virgineus*.

(i) *List of species characteristic of grasslands.*

A complete list of all the species found is not given, but below will be found a list of all the so-called "constant" species arranged in order of descending constancy. In this case the two years are added together, therefore 20 is the highest possible constancy value.

Constancy 100–80%

Hygrophorus coccineus, H. psittacinus, H. virgineus, Mycena metata, Naucoria melinoides, Panaeolus campanulatus, P. papilionaceus, Stropharia semiglobata.

Constancy 80–60%

Clitocybe rivulosa, Galera hypnorum, G. tenera, Hygrophorus chlorophanus, H. pratensis, H. virgineus var. *roseipes, Lycoperdon depressum, Mycena ammoniaca, Psaliota campestris, Psilocybe semilanceata, Stropharia aeruginosa, Tubaria furfuracea.*

Constancy 60–40%

Clavaria corniculata, C. fusiformis, Clitocybe fragrans, Coprinus niveus, C. plicatilis, Entoloma sericeum, Hygrophorus ceraceus, H. conicus, H. ovinus, Lepiota granulosa, Lycoperdon perlatum, Mycena avenacea, M. epipterygia, M. pelliculosa, Nolanea proletaria, Omphalia fibula, Psathyrella atomata.

Constancy 40–20%

Anellaria separata, Bolbitius fragilis, B. vitellinus, Clavaria dissipabilis, Clitocybe aurantiaca var. *nigripes, C. infundibuliformis, Collybia butyracea, Cortinarius anomalus, C. erythrinus, Entoloma jubatum, Galera ovalis, Hebeloma crustuliniforme, H. crustuliniforme* var. *minus, Hygrophorus Colemannianus, H. laetus, H. miniatus, H. puniceus,*

H. turundus, H. unguinosus, Lepiota procera, Leptonia solstitialis, Lycoperdon pusillum, Mycena flavo-alba, Naucoria cerodes, N. pusiola, Omphalia fibula var. *Schwartzii, Panaeolus sphinctrinus, Psaliota campestris* var. *rufescens, Psathyra corrugis, P. fatua, Stropharia inuncta, Tricholoma cuneifolium, T. melaleucum, T. sordidum.*

As will be seen later several of the last class are to be found exclusively, or for the greater part, on one only of the soil types.

For the sake of interest the constancy figures were also calculated for the two years separately, i.e. giving a maximum constancy of 40. As might be expected, owing to the similarity between the two years, this second list was very like the first. In general, the relative position of the species in order of constancy remained unchanged, the main difference being that with the larger number of stations the constancy values were on the whole lower. Thus there were only fifty species in the "over 20%" class as compared with the previous seventy-one species.

(ii) *Constancy in relation to soil type.*

Previous work (Wilkins *et al.* 1938) indicated that the soil has an important influence on the distribution of fungi in woodlands, and this influence is equally important in the case of grassland fungi. Table VI gives the numbers of species found on each station, the stations being grouped under the three headings chalk, clay and sand. The two years 1936 and 1937 are given separately and together.

Table VI. *Numbers of species on three soil types*

	1936	Average	1937	Average	1936 and 1937	Average
Chalk:						
White Horse Hill	27	39	22	31	37	53
Waltham Meadows	37		13		39	
Blenheim Park	51		29		64	
Chinnor Hill	26		36		47	
Crowell Hill	42		38		59	
Graffham Down	40		34		58	
Heyshott Down	50		48		67	
Clay:						
Langton Meadows	18	18	6	18	21	29
Evington Meadows	22		8		25	
Horspath Meadows	16		33		40	
Waterperry Common	12		10		16	
Frillinghurst Meadows	17		23		32	
Gospel Green	25		26		42	
Sand:						
Donington Meadows	41	37	15	35	45	50
Rothley Meadows	26		18		33	
Nuneham Park	46		40		63	
North Heath	35		50		60	
Bradgate Park	36		31		44	
Valewood Meadows	40		48		58	
Turville Heath	37		40		49	

Table VI shows that there is no significant difference between the two years, either in the average number of species collected in each year or in the proportional distribution on the three soil types. There is little difference between the species numbers on chalk and sand, but clay gives only about one-half the number of either of the other two. The total number of species found on each soil type tends to confirm this (Table VII).

Table VII. *Total number of species on each soil type*

	1936	1937	1936 and 1937
Chalk	115	93	139
Clay	51	51	70
Sand	80	86	107

Here it seems that chalk shows a rather higher total number of species than sand and, as before, twice as many as clay. The fact that the total number on chalk is greater than on sand while the average numbers per station are similar, indicates that there is greater variation between the individual stations on chalk than between those on sand. This is confirmed by a comparison between the species constancy figures for the three soil types (Table VIII).

Table VIII. *Constancy figures for each of the three soil types*

Chalk

14	13	12	11	10	9	8	7	6	5	4	3	2	1
—	2	3	2	2	4	5	5	7	11	10	13	21	54
							13%					87%	

Clay

12	11	10	9	8	7	6	5	4	3	2	1
1	—	2		1	5	3	6	5	5	16	26
					13%				87%		

Sand

14	13	12	11	10	9	8	7	6	5	4	3	2	1
4	3	4	4	2	3	6	4	4	8	7	13	16	29
			24%							76%			

The equal division of the constancy classes shows that in the highest class the species on chalk and clay are equally constant at 13%, but that the species on sand have a degree of constancy about twice greater than either, i.e. 24%.

(iii) *List of species characteristic of each soil type.*

Below will be found a complete list of the species which are characteristic of each soil type. The list is arranged in columns showing the comparative constancy in descending order.

Chalk	Clay	Sand
	100–80 %	
Hygrophorus Colemannianus	*Hygrophorus virgineus*	*Clavaria dissipabilis*
H. virgineus	*Psilocybe semilanceata*	*C. fusiformis*
Lycoperdon perlatum	*Stropharia semiglobata*	*Hygrophorus chlorophanus*
Naucoria melinoides		*H. coccineus*
Tricholoma melaleucum		*H. pratensis*
		H. psittacinus
		H. virgineus
		Naucoria melinoides
		Panaeolus campanulatus
		Psilocybe semilanceata
		Stropharia semiglobata
	80–60 %	
Clitocybe fragrans	*Psaliota campestris*	*Hygrophorus ceraceus*
C. rivulosa		*H. unguinosus*
Galera hypnorum		*H. virgineus* var. *roseipes*
Hygrophorus coccineus		*Mycena ammoniaca*
Lycoperdon depressum		*M. metata*
Panaeolus campanulatus		*Nolanea proletaria*
Stropharia aeruginosa		*Omphalia fibula*
S. inuncta		*Panaeolus papilionaceus*
		Stropharia aeruginosa
	60–40 %	
Clavaria corniculata	*Clitocybe fragrans*	*Clavaria corniculata*
Clitocybe infundibuliformis	*Coprinus niveus*	*Coprinus niveus*
Coprinus plicatilis	*C. plicatilis*	*Entoloma jubatum*
Cortinarius anomalus	*Galera tenera*	*E. sericeum*
Entoloma sericeum	*Hygrophorus chlorophanus*	*Galera hypnorum*
Galera tenera	*H. coccineus*	*Hygrophorus laetus*
Hebeloma crustuliniforme var. *minus*	*H. psittacinus*	*H. miniatus*
	Lycoperdon depressum	*H. ovinus*
Hygrophorus ovinus	*Mycena ammoniaca*	*H. puniceus*
H. virgineus var. *roseipes*	*M. metata*	*Lepiota granulosa*
Lepiota gracilenta	*Naucoria melinoides*	*Lycoperdon nigrescens*
L. granulosa	*Panaeolus campanulatus*	*L. perlatum*
Mycena epipterygia	*P. papilionaceus*	*Mycena avenacea*
M. metata	*Tubaria furfuracea*	*M. epipterygia*
Panaeolus papilionaceus		*Tubaria furfuracea*
Psaliota campestris		
Psathyrella atomata		
Stropharia semiglobata		
Tricholoma cuneifolium		
	40–20 %	
Astrosporina asterospora	*Clavaria corniculata*	*Anellaria separata*
Bolbitius fragilis	*Clitocybe rivulosa*	*Bolbitius vitellinus*
Clitocybe cyathiformis	*Coprinus niveus*	*Clavaria fragilis*
C. nebularis	*Entoloma sericeum*	*C. vermicularis*
Collybia butyracea	*Galera hypnorum*	*Clitocybe aurantiaca* var. *nigripes*
Cortinarius cinnamomeus	*Hygrophorus conicus*	
C. erythrinus	*H. pratensis*	*C. fragrans*
Geoglossum glabrum	*H. unguinosus*	*C. rivulosa*
Hygrophorus ceraceus	*Nolanea proletaria*	*Collybia butyracea*

Chalk	Clay	Sand
	40–20%	
Hygrophorus chlorophanus	*Psaliota campestris* var.	*Coprinus plicatilis*
H. conicus	*rufescens*	*Cortinarius erythrinus*
H. pratensis	*Psathyrella atomata*	*Entoloma prunuloides*
H. psittacinus		*Galera tenera*
H. turundus		*Hygrophorus conicus*
Inocybe cervicolor		*H. irrigatus*
Lepiota clypeolaria		*Lepiota procera*
Leptonia sericella		*Leptonia solstitialis*
Lycoperdon excipuliforme		*Lycoperdon depressum*
L. pusillum		*L. pusillum*
L. umbrinum		*Mycena pelliculosa*
Mycena avenacea		*Naucoria pusiola*
M. flavo-alba		*Omphalia fibula* var.
M. pelliculosa		*Schwartzii*
M. pura		*Panaeolus sphinctrinus*
Naucoria badipes		*Psaliota campestris*
N. cerodes		*P. campestris* var. *rufescens*
N. pusiola		*Psilocybe ericaea*
Omphalia fibula		*Scleroderma aurantium*
Psaliota dulcidula		*Tricholoma columbetta*
Tricholoma bufonium		*T. sordidum*
T. humile		
T. irinum		
T. sordidum		
Tubaria furfuracea		

(*b*) *Frequency of individuals*

It has been shown (Wilkins *et al.* 1937) that the mycological flora of any given community should be expressed in terms of the relative constancy of species in the different stations and, also, by the frequency of individuals in these stations. In this paper, frequency has been considered in connexion with grasslands in general and, also, in relation to grasslands of the respective soil types.

(i) *List of grassland species.*

The following list gives those species with a frequency above 10 which have been found on all stations during the two years.

Over 2000 individuals

Hygrophorus coccineus, H. psittacinus, H. virgineus, Naucoria melinoides, Psilocybe semilanceata, Stropharia semiglobata.

2000–1500 individuals

Clavaria fusiformis, Clitocybe nebularis, Hygrophorus chlorophanus, Mycena metata, Nolanea proletaria, Panaeolus campanulatus.

1500–1000 individuals

Clitocybe rivulosa, Galera hypnorum, Hygrophorus conicus, H. virgineus var. *roseipes.*

1000–500 individuals

Clavaria dissipabilis, Hygrophorus pratensis, H. unguinosus, Mycena epipterygia, Omphalia fibula, Panaeolus papilionaceus.

500–50 individuals

Clavaria corniculata, C. vermicularis, Clitocybe fragrans, C. infundibuliformis, Coprinus niveus, C. plicatilis, Cortinarius erythrinus, Entoloma jubatum, E. prunuloides, E. sericeum, Galera tenera, Hygrophorus ceraceus, H. Colemannianus, Lepiota granulosa, Lycoperdon depressum, L. nigrescens, L. perlatum, Mycena ammoniaca, M. avenacea, Psaliota campestris, Stropharia aeruginosa, S. inuncta, Tricholoma bufonium, T. irinum, T. melaleucum, T. personatum, Tubaria furfuracea.

50–10 individuals

Anellaria separata, Astrosporina asterospora, Clavaria fragilis, C. tenuipes, Clitocybe aurantiaca var. *nigripes, C. clavipes, C. cyathiformis, C. dealbata, Clitopilus popinalis, C. prunulus, Collybia butyracea, Cortinarius anomalus, Geoglossum glabrum, Hebeloma crustuliniforme, H. crustuliniforme* var. *minus, Hygrophorus irrigatus, H. laetus, H. miniatus, H. nitratus, H. ovinus, H. puniceus, H. turundus, Lepiota gracilenta, L. procera, Leptonia solstitialis, Lycoperdon pusillum, L. umbrinum, Marasmius erythropus, M. oreades, Mycena flavo-alba, M. pelliculosa, M. pura, Naucoria badipes, N. cerodes, N. pusiola, Omphalia fibula* var. *Schwartzii, Panaeolus sphinctrinus, Psaliota campestris* var. *rufescens, P. dulcidula, Psathyra corrugis, P. fatua, Psathyrella atomata, Psilocybe ericaea, Tricholoma carneum, T. cuneifolium, T. humile, T. panaeolum* var. *caespitosum, T. sordidum, T. terreum* var. *atrosquamosum.*

The above list confirms in a general sense the constancy list, in that species with relatively high frequency values usually have high constancy values for the 20 stations investigated. Table IX brings out this point.

Table IX. *General comparison between frequency and constancy*

No. of species	Frequency	Average % constancy
6	+2000	87
5	2000–1500	70
4	1500–1000	65
6	1000– 500	53
27	500– 50	41
49	50– 10	22

One species, *Clitocybe nebularis*, has been omitted as it forms a very definite exception in that, though its frequency value was very high, its constancy value was almost negligible since it occurred on only two stations, both on chalk. With this exception, the above list shows remarkable correlation between frequency and constancy and emphasizes the point that both these factors should be considered in estimating the distribution of fungi.

(ii) *Frequency in relation to soil types.*

In round figures the approximate number of individuals found on each of the soil types was:

Chalk	10,000
Clay	5,000
Sand	40,000

These figures are an approximate estimate of the actual number of sporophores on the areas investigated, but they indicate the proportional distribution of individuals in the three soil types. By comparison with Table VII, which showed the number of species on each soil type, it will be seen that, though the numbers of species on chalk and sand are approximately of the same order and are twice as many as on clay, the number of individuals on chalk is still twice as many as on clay but only a quarter of those found on sand. That there is no correlation between numbers of species and numbers of individuals on the three soil types is shown by the following ratios:

	Chalk		Clay		Sand
Species	2	:	1	:	2
Individuals	2	:	1	:	8

(iii) *List of species characteristic of each soil type.*

The following is a complete list of the species found on each soil type, arranged in order of descending frequency.

Chalk	Clay	Sand
	Over 2000 sporophores	
		Hygrophorus coccineus
		H. virgineus
		Psilocybe semilanceata
		Stropharia semiglobata
	2000–1500 sporophores	
Clitocybe nebularis		*Clavaria fusiformis*
		Hygrophorus chlorophanus
		H. psittacinus
		Nolanea proletaria
	1500–1000 sporophores	
Hygrophorus virgineus		*Hygrophorus virgineus* var. *roseipes*
		Mycena metata
		Naucoria melinoides
		Panaeolus campanulatus
	1000–500 sporophores	
Clitocybe rivulosa	*Panaeolus campanulatus*	*Clavaria dissipabilis*
Galera hypnorum	*P. papilionaceus*	*Hygrophorus conicus*
Hygrophorus conicus		*H. pratensis*
Mycena epipterygia		*H. unguinosus*
Naucoria melinoides		*Omphalia fibula*

Chalk	Clay	Sand
500–50 sporophores		
Clitocybe fragrans	*Coprinus niveus*	*Clavaria corniculata*
C. infundibuliformis	*Hygrophorus chlorophanus*	*C. vermicularis*
Coprinus niveus	*H. coccineus*	*Clitocybe rivulosa*
Entoloma sericeum	*H. psittacinus*	*Coprinus niveus*
Hygrophorus coccineus	*H. virgineus*	*Entoloma jubatum*
H. Colemannianus	*Mycena ammoniaca*	*E. prunuloides*
H. unguinosus	*M. metata*	*E. sericeum*
Lycoperdon depressum	*Nolanea proletaria*	*Galera hypnorum*
L. perlatum	*Psilocybe semilanceata*	*G. tenera*
Mycena metata	*Stropharia semiglobata*	*Lepiota granulosa*
Panaeolus campanulatus	*Tricholoma personatum*	*Lycoperdon nigrescens*
P. papilionaceus		*Mycena ammoniaca*
Stropharia aeruginosa		*M. avenacea*
S. inuncta		*M. epipterygia*
Tricholoma bufonium		*Panaeolus papilionaceus*
T. irinum		*Stropharia aeruginosa*
T. melaleucum		*Tubaria furfuracea*
Tubaria furfuracea		
50–10 sporophores		
Astrosporina asterospora	*Clavaria corniculata*	*Anellaria separata*
Clavaria corniculata	*C. fusiformis*	*Clavaria fragilis*
C. fusiformis	*Clitocybe fragrans*	*C. tenuipes*
Clitocybe cyathiformis	*C. rivulosa*	*Clitocybe aurantiaca* var. *nigripes*
Coprinus plicatilis	*Coprinus plicatilis*	*C. clavipes*
Cortinarius anomalus	*Entoloma jubatum*	*C. dealbata*
C. erythrinus	*E. sericeum*	*C. fragrans*
C. hinnuleus	*Galera hypnorum*	*C. infundibuliformis*
Galera tenera	*G. tenera*	*Clitopilus popinalis*
Geoglossum glabrum	*Hebeloma crustuliniforme* var. *minus*	*C. prunulus*
Hebeloma crustuliniforme	*Hygrophorus pratensis*	*Collybia butyracea*
H. crustuliniforme var. *minus*	*H. virgineus* var. *roseipes*	*Coprinus plicatilis*
Hygrophorus ceraceus	*Lycoperdon depressum*	*Cortinarius erythrinus*
H. ovinus	*Marasmius oreades*	*Hygrophorus irrigatus*
H. pratensis	*Mycena avenacea*	*H. laetus*
H. psittacinus	*M. flavo-alba*	*H. miniatus*
Lepiota gracilenta	*Naucoria melinoides*	*H. nitratus*
L. granulosa	*Omphalia fibula*	*H. puniceus*
Lycoperdon pusillum	*Panaeolus sphinctrinus*	*H. turundus*
L. umbrinum	*Psaliota campestris*	*Lepiota procera*
Mycena ammoniaca	*P. campestris* var. *rufescens*	*Leptonia solstitialis*
M. avenacea	*Psathyra fatua*	*Lycoperdon depressum*
M. pelliculosa	*Tricholoma panaeolum* var. *caespitosum*	*L. perlatum*
M. pura	*Tubaria furfuracea*	*L. pusillum*
Naucoria badipes		*Marasmius erythropus*
N. cerodes		*Mycena pelliculosa*
Omphalia fibula		*Naucoria cerodes*
Psaliota campestris		*N. pusiola*
P. dulcidula		*Omphalia fibula* var. *Schwartzii*
Psathyra corrugis		*Panaeolus sphinctrinus*
P. fatua		*Psaliota campestris* var. *rufescens*
Psathyrella atomata		*Psathyra fatua*
Stropharia semiglobata		*Psathyrella atomata*
Tricholoma cuneifolium		*Psilocybe ericaea*
T. humile		*Tricholoma carneum*
T. sordidum		*T. sordidum*
T. terreum var. *atrosquamosum*		

(c) Comparison of constancy and frequency in certain species

It has been shown that, in general, a species which is abundant is also relatively constant. When considering the three soil types, however, it is found that even when constancy on all stations is high and a species is generally abundant there may be a marked preference for one or two of the soil types. Table X shows some of the more important preferences. The table is arranged on the basis of constancy in each of the soil types with the frequency figures added as a comparative factor. It must be understood that the term "relative" has been allowed generous latitude. Species marked with an asterisk are exclusive.

Table X. *Comparison of constancy and frequency in the three soil types in the case of certain species*

	Constancy			Frequency		
	Chalk	Clay	Sand	Chalk	Clay	Sand
A. Species with relatively equal distribution						
Entoloma sericeum	50	33	36	124	22	320
Galera tenera	43	42	36	42	32	113
Hygrophorus coccineus	71	50	80	127	303	2142
H. conicus	36	33	30	504	112	512
H. virgineus	86	83	100	1181	64	2330
H. virgineus var. *roseipes*	57	33	79	260	31	1140
Mycena metata	57	58	71	152	132	1440
Naucoria melinoides	86	50	86	552	24	1451
Panaeolus campanulatus	64	66	86	216	534	1231
Psaliota campestris	50	66	30	16	44	4
Stropharia semiglobata	57	83	100	17	460	2352
Tubaria furfuracea	36	50	57	113	24	53
B. Species whose constancy is relatively high on chalk and sand						
Galera hypnorum	64	25	57	742	22	332
Hygrophorus ovinus	50		50	15	-	7
Lepiota granulosa	43	—	57	24	—	53
Lycoperdon perlatum	93	—	50	364	—	43
L. pusillum	36	—	21	14	—	12
Mycena epipterygia	57	-	43	525	—	60
M. pelliculosa	29		36	13		32
Stropharia aeruginosa	79	17	64	155	2	63
Tricholoma sordidum	36	8	29	23	1	22
C. Species whose constancy is relatively high on clay and sand						
Coprinus niveus	7	42	43	100	131	213
Hygrophorus chlorophanus	21	58	93	3	61	1762
H. psittacinus	36	57	93	14	223	1843
Mycena ammoniaca	14	42	79	20	122	182
Psilocybe semilanceata	14	100	100	2	444	3660

Table X (*contd.*)

	Constancy			Frequency		
	Chalk	Clay	Sand	Chalk	Clay	Sand
D. Species whose constancy is relatively high on chalk						
Clitocybe infundibuliformis	50	17	7	142	2	10
C. rivulosa	79	33	36	852	22	131
**Cortinarius anomalus*	57	—	—	26	—	—
Hebeloma crustuliniforme var. *minus*	43	8	7	33	10	1
**Hygrophorus Colemannianus*	86	—	—	93	—	—
**Inocybe cervicolor*	36	—	—	5	—	—
Lepiota gracilenta	43	—	7	24	—	1
**Mycena pura*	36	—	—	14	—	—
Psathyrella atomata	50	25	7	34	3	10
**Stropharia inuncta*	71	—	—	55	—	—
**Tricholoma bufonium*	36	—	—	122	—	—
T. cuneifolium	43	8	7	24	1	1
**T. melaleucum*	93	—	—	175	—	—
E. Species whose constancy is relatively high on sand						
Clavaria dissipabilis	7	—	86	1	—	943
C. fusiformis	14	17	100	11	11	1781
Entoloma jubatum	—	17	57	—	20	53
Hygrophorus ceraceus	28	17	71	22	2	253
**H. laetus*	—	—	50	—	—	43
**H. miniatus*	—	—	43	—	—	24
H. pratensis	29	25	93	13	21	943
**H. unguinosus*	—	—	64	—	—	733
**Lycoperdon nigrescens*	—	—	60	—	—	332
Nolanea proletaria	7	25	79	1	111	1832
Omphalia fibula	21	17	64	21	11	634
Panaeolus papilionaceus	43	42	79	105	522	209

Comparison between constancy and frequency as illustrated in Table X reveals interesting points:

(1) It appears from section A that frequency of individuals in each of the soil types is more variable than constancy, as no species is equally abundant on all three types though it may be relatively constant on all three.

(2) In the case of those species whose constancy figures show a definite preference for one or two of the soil types, the frequency figures tend to emphasize this point, as shown in sections B, C, D and E.

(3) In certain cases where species show relatively high constancy on two stations, the frequency figures show a definite preference for one of these, e.g. in section B, *Lycoperdon perlatum*, *Mycena epipterygia* and *Galera hypnorum*, and in section C, *Hygrophorus chlorophanus* and *Psilocybe semilanceata*.

(4) In section E, *Panaeolus papilionaceus* is an exception to the general rule, given in (2) above, in that the constancy is high on sand while the frequency is highest on clay.

A consideration of all the facts referring to the distribution of species in relation to soil shows that, within the somewhat limited scope of the investigation, certain genera show definite preferences for one or other of the soil types, while others are very tolerant of soil conditions. For instance, the genera *Clitocybe* and *Tricholoma* were found predominantly on chalk, and all the five species of *Clavaria* were found on sand either exclusively or with a higher degree of constancy than elsewhere. Of the genera which were found on all types of soil, there is variation in the degree to which individual species show a high or low constancy on the different soil types. This is well illustrated by the genus *Hygrophorus*. The more or less general distribution of the genera *Bolbitius*, *Coprinus* and *Panaeolus* is probably due to their coprophilous habit, rendering them to some extent independent of the soil conditions.

VI. Discussion

It would seem that the above recorded differences in the distribution of grassland fungi are mostly due to edaphic factors. Illumination, which may have an influence in woodlands, can, in the present case, be regarded as constant, and factors such as elevation, slope, etc., have been largely counterbalanced by taking a number of differently situated stations. There appeared to be no correlation between organic content and abundance of sporophores, as the organic content of chalk, clay and sand was respectively 21·9, 11·2 and 9·3%, while the number of sporophores on the three was 10,000, 5000 and 40,000. The striking feature was the relatively few species and individuals found on the clay soils. This is probably consequent upon lack of aeration due to waterlogging, especially in winter. It was noted that certain areas on clay which were subject to periodic flooding were devoid of fungal species. The clay soils having a higher specific heat are slow to lose heat in autumn, but it was noticed that the fungi did not continue later in the autumn on the clay soils than on either of the other soils.

The *p*H of the soil may have some effect on fungus distribution. Fungi on the whole prefer a slightly acid substrate, and the absence of calcium carbonate in the sand soils may be favourable. This suggestion is to some extent confirmed by an observation on Chinnor Hill, where the partially leached plateau with a *p*H of 5·9–6·5 had a much more abundant fungus flora than had the escarpment slope with a *p*H of 7·5. It is probable that the distribution of such species as *Psilocybe semilanceata*, with a constancy/frequency of 14/100, 100/2, 444/3660 on chalk, clay and

sand, may be due to intolerance of alkaline conditions. In contrast to this there are several species which are found exclusively or preferentially on alkaline soils.

There is a possibility that the phanerogamic species may influence fungus distribution. It has been shown that several grasses such as *Festuca ovina*, *Holcus lanatus*, *Lolium temulentum*, *L. multiflorum*, *L. rigidum* and *L. strictum* are mycorrhizal, though in all these cases the fungus has been either a Phycomycete or an Ascomycete, and no work has demonstrated the significance of the Basidiomycetes in this connexion. The direct association between the grassland vegetation and the fungi is a matter of conjecture, but two instances of a positive nature may be mentioned. *Cortinarius anomalus* was found on the chalk soils only, and with a constancy of 57%. It was invariably growing among or beside plants of *Helianthemum vulgare*, which was itself found only on chalk grasslands. On White Horse Hill the *Helianthemum* was present only as three clumps and *Cortinarius anomalus* was found growing in two of these but nowhere else on the station. *Helianthemum* was locally abundant on Heyshott and Graffham Downs, and *Cortinarius anomalus* was frequently associated with it, though never found elsewhere. Similarly, *Tricholoma melaleucum*, also exclusive to chalk, was invariably in proximity to *Carex glauca*. The limited number of observations excludes definite conclusion, but suggests investigation.

As would be expected from the fact that these grasslands can be regarded as of one ecological type in spite of soil variation, there is a reasonable uniformity of fungus population. Excluding the sporadics, i.e. those below 20% constancy, of the remaining sixty-four species forty-four are found on all three soil types, seventeen upon two, and only eight are exclusive to one soil type.

Comparison between the fungus flora of grasslands and that of oakwoods shows that there is considerable difference both in actual species and in numbers of species. Out of 172 species on twenty grasslands and 469 species in twenty oakwoods, only 86 species were common. Of these, forty-five were sporadic on grassland while fifty-five were sporadic in oakwoods, leaving only seventeen species with a constancy over 20% common to both communities. These species are:

Clavaria fusiformis, *Coprinus plicatilis*, *Cortinarius erythrinus*, *Galera hypnorum*, *Hebeloma crustuliniforme*, *H. crustuliniforme* var. *minus*, *Hygrophorus coccineus*, *H. psittacinus*, *H. virgineus*, *Lycoperdon perlatum*, *Mycena ammoniaca*, *M. epipterygia*, *Omphalia fibula*, *Psathyrella atomata*, *Stropharia aeruginosa*, *S. semiglobata*, *Tubaria furfuracea*.

The dissimilarity between the fungi of grasslands and those of beechwoods is even greater. Out of 172 species of grassland fungi and a total of 419 in beechwoods, only seventy species were common. Of these, thirty-seven were sporadic in grasslands and forty seven were sporadic in beechwoods, leaving only the following eleven species, with a constancy of over 20%, common to the two communities:

Clitocybe infundibuliformis, Clitopilus prunulus, Collybia butyracea, Coprinus plicatilis, Galera hypnorum, Hebeloma crustuliniforme, Lycoperdon perlatum, Mycena ammoniaca, Naucoria melinoides, Omphalia fibula, Stropharia aeruginosa.

Not only are the individual species different in the grass/oak and grass/beech communities, but the dominant genera are not the same. *Hygrophorus* is the representative genus of grasslands with, in this investigation, eighteen species of which twelve have a constancy above 20%, whereas of the fifteen species found in oakwoods thirteen are sporadic, and of the nine species in beechwoods eight are sporadic. On the other hand, the typical woodland genera, *Boletus, Lactarius, Russula,* etc., are not found in grassland. It must be understood that in both cases the stations were somewhat heterogeneous. Though an attempt was made to exclude certain species from grassland when they were obviously associated with scattered trees (p. 32) no such allowance was made for the grassy rides in oakwoods and beechwoods. Had this been done, the respective mycological floras would undoubtedly have been even more characteristic.

The actual number of species found on grasslands is small compared with those in oakwoods and beechwoods. This is accounted for by the fact that, whereas the oakwoods and beechwoods were examined over a period of six years by a number of people, the grasslands were only investigated for two years largely single handed. Apart from this, however, the severer climatic conditions to which the open grasslands are subjected limit the number of fungi. It has been noticed that a season in which fungi were abundant in woodlands produced comparatively few species on grasslands. There is, in the case of grasslands, a greater variation of temperature, with a corresponding drying out of available moisture, and the fungus season is comparatively short. The influence of climatic factors on grassland fungi is at present being investigated.

VII. Summary

1. This paper is an investigation into the mycological flora characteristic of grasslands with particular reference to certain soil types.

2. Twenty stations were examined over a period of two years, the stations being representative of chalk, clay and sand soils.

3. The experimental method is briefly described and ecological notes on the twenty stations are given in detail.

4. Lists of the fungi found on grasslands in general and on each of the three soil types are given, and the constancy of species and frequency of individuals are discussed in relation to grasslands as a whole and in relation to each soil type.

5. The results are discussed and a comparison made between the fungi of grasslands and those of oakwoods and beechwoods.

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References

Gilbert, E. J. (1928). *La mycologie sur le terrain.* Paris.

Graham, V. O. (1927). Ecology of the fungi in the Chicago region. *Bot. Gaz.* **82**, 267.

Hooker, Sir J. D. (1930). *The Students' Flora of the British Isles.* London.

Rea, Carlton (1922). *British Basidiomycetae.* Cambridge.

Walkley, A. & Black, I. A. (1934). An examination of the Degltareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**, 29.

Wilkins, W. H., Ellis, E. M. & Harley, J. L. (1937). The ecology of the larger fungi. I. Constancy and frequency of fungal species in relation to certain vegetation communities particularly oak and beech. *Ann. appl. Biol.* **24**, 703.

Wilkins, W. H., Harley, J. L. & Kent, G. C. (1938). The ecology of the larger fungi. II. The distribution of the larger fungi in part of Charlton Forest, Sussex. *Ann. appl. Biol.* **25**, 472.

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SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT

IV. FACTORS LIMITING INFECTION BY ASCOSPORES OF *OPHIOBOLUS GRAMINIS*

By S. D. GARRETT

Rothamsted Experimental Station, Harpenden, Herts

(With Plate III)

CONTENTS

I. Introduction

After finding that the ascospores of *Ophiobolus graminis* are ejected from their perithecia into the air, Samuel & Garrett (1933) suggested that aerial dispersal of ascospores in showery weather might be responsible for the widespread epidemics of the take-all disease that occurred in South Australia in seasons of good spring rainfall. It was considered that the late appearance of the disease in the whiteheads form over whole fields of wheat might be due to infection from ascospores produced on plants killed early. The ascospore dispersal hypothesis thus seemed to account not only for the sudden appearance of whiteheads at heading time in crops that had shown little sign of the disease earlier, but also for the apparent limitation of widespread outbreaks of the disease to years of good spring rainfall in South Australia (Garrett, 1934).

Subsequent work (Garrett, 1936) on the subterranean activity of *O. graminis* on the wheat root-system has rendered an alternative explanation increasingly probable, though not necessarily to the exclusion of the ascospore dispersal hypothesis. This later work has shown that *Ophiobolus* spreads underground along the wheat roots at a rate which varies greatly with the nature and condition of the soil. If the amount of infectious material surviving in the soil from a previous diseased crop be

sufficient, a severe attack of the disease may occur on any soil and in any season. But with a sparser distribution of infectious material in the soil, the production of whiteheads will follow only on such soils and in such seasons as are favourable to the subterranean spread of the fungus. Thus, these general outbreaks of whiteheads occurred in South Australia only on the light-textured alkaline soils of the "mallee" areas, and only in years of good spring rainfall. Such soils, when the moisture content is adequate, have been shown to be very favourable to the spread of *Ophiobolus* along the roots (Garrett, 1934); in dry seasons, however, the drying-out of the surface soil may be expected to check the mycelial advance of the fungus towards the crown of the plant, and hence to retard or prevent altogether the production of whiteheads. Soil moisture is more likely to act as a factor limiting mycelial advance in South Australia than in England, where soil moisture content is generally higher throughout the growing season.

Gradual subterranean spread of the fungus from numerous scattered foci of infection can probably thus account, equally well with the hypothesis of ascospore dispersal, for these two features of take-all outbreaks in South Australia, viz. the unexpected development of whiteheads in full-sized plants with the advent of the first hot ripening weather, and limitation to seasons of good spring rainfall. An adequate investigation of ascospore infection is desirable in order that the relative importance of soil infection and ascospore infection may be assessed, and control measures directed accordingly. This need has recently been emphasized by Samuel (1937), in discussing the field occurrence of the disease in England.

II. Experimental

Perithecia of *O. graminis* were obtained in abundance by the following method. Wheat seedlings were planted above agar inoculum of the fungus in 2·8 × 20 cm. boiling tubes, filled to a height of 8 cm. with sand moistened with a standard nutrient solution for wheat plants. The sand was brought to a moisture content of 50% saturation by the addition of this nutrient solution (75 g. dry sand and 12 c.c. solution per tube). Five wheat seeds were planted over an agar inoculum disk of *Ophiobolus*, and covered with moist sand. The tubes were plugged with cotton-wool and incubated in glass jars kept at laboratory temperature (16–20° C.) in a north window. Perithecia usually began to form on the infected roots and stems exposed to the light after a period of 2–3 weeks; mature ascospores could generally be obtained from such perithecia after 6 weeks.

The success of this method may be attributed to the provision of the natural substrate, an atmosphere of sufficient humidity, an adequate light intensity, and to the fact that, deliberately, other micro-organisms were not excluded. The stimulating effect of other micro-organisms upon the production of the perfect stage of fungi growing upon agar may frequently be observed, and has been investigated critically by Asthana & Hawker (1936), Hawker (1936) and others. From the ripe perithecia produced in these tubes, a suspension of ascospores could be obtained by soaking the perithecia-bearing stems and roots in water, and then straining the resulting suspension of spores through muslin. Smaller numbers of ascospores in bacteriologically sterile suspension could be obtained conveniently by allowing moistened ripe perithecia to eject their ascospores on to sterile coverslips $\frac{1}{2}$ 1 mm. above the necks, as already described by Samuel & Garrett (1933).

No difficulty in obtaining ascospore infection had been anticipated, since mature ascospores always germinated readily on nutrient agars, and infection could always be obtained with the resulting agar cultures. Many of the isolates employed in preceding work with the fungus were derived originally from ascospores. It was, therefore, surprising to find that a series of infection experiments with ascospores gave completely negative results. Ascospores obtained as described above, and employed as soon as mature, generally showed a germination of more than 90% on 0·5% dextrose agar, yet infection failed to occur on a variety of soil types, either with germinating seeds or with seedlings of different ages. No infection was obtained, again, in pure sand, or in sand with an admixture of 1/3 or 1/4 of a steamed alkaline soil, known to be especially favourable to the spread of the fungus along the roots. The majority of infection experiments were conducted with a suspension of some 30,000 ascospores per c.c., of which 1 c.c. was added to the soil around each seedling, or to each planting hole for germinating seeds. In one experiment, 1 c.c. of a suspension containing 80,000 ascospores per c.c. was used per planting hole, but with equal lack of success. Infection of the roots of wheat seedlings by *Ophiobolus* is normally revealed by discoloration of the tissues within one week (at 20° C.) of inoculation. Roots of the ascospore-inoculated seedlings were searched for lesions and for the presence of runner hyphae of *Ophiobolus* under the binocular dissecting microscope, at a period of 2–3 weeks after inoculation, but without success. Ascospore-inoculated plants were also grown for a period of 2 months in the glasshouse, but the longer period of incubation again failed to reveal any trace of infection.

Since ascospores of *Ophiobolus* used in these experiments showed a

germination exceeding 90% on 0·5% dextrose agar, the mere increase of density in the spore suspension used for inoculation did not seem likely to lead to infection. Consideration of the fact that a nutrient agar culture derived from the ascospores would always give infection led to realization that the factor limiting ascospore infection might possibly be a nutritive one. Thus Brown (1922) found that spores of *Botrytis cinerea*, if sown in water on the healthy, uninjured leaf of broad bean, were unable to cause infection; if they were sown in nutrient solution (turnip extract), infection occurred at once. Brown further demonstrated a relationship between exosmosis of solutes from uninjured flower petals of different species, as measured by the conductivity of the infection drop, and their susceptibility to infection by the spores of *Botrytis*. It appeared possible that the ascospores of *Ophiobolus* might be able to cause infection of the seedling roots if a suitable extraneous nutrient were provided. One-half per cent of glucose was therefore added to the ascospore suspension in several of the infection experiments, but infection still failed to occur.

The analogy between spore infection by a leaf-infecting fungus such as *Botrytis* and a root-infecting fungus such as *Ophiobolus* is incomplete, however, inasmuch as spore germination and root infection by *Ophiobolus* are likely to be greatly impeded, if not inhibited, by the competition and antagonism of saprophytes developing on the glucose or other nutrient material added. For the production of conditions as favourable to infection as those obtaining in the *Botrytis* infection drop on the leaf, it would be necessary not only to present the ascospores of *Ophiobolus* with an accessary nutrient, but also to protect this nutrient from the rapid development of other soil micro-organisms. Sterilized soil is an environment satisfying both these requirements, for it serves as a suitable culture medium for *Ophiobolus* without the addition of further nutrients than those already set free by the process of heat sterilization.

An experiment was, therefore, set up to test sterilized soil as an infection medium for the ascospores of *Ophiobolus*. The soil employed was from Bridgham, Norfolk—a very light sandy loam of the Breckland type, overlying chalk and well supplied with calcium carbonate (*p*H value of soil approx. 8·0). This soil had previously been found very favourable to spread of the fungus along the roots, on account of its physical and chemical properties (Garrett, 1936). The experiment was conducted in large test tubes, 2·0 × 17·5 cm., which were filled to a depth of 6·5 cm. by the addition of 28 g. air-dry Bridgham soil; 5 c.c. of distilled water was then added to each tube to bring the soil to a moisture content of approximately 50% saturation. Two dozen tubes were thus filled with soil,

plugged with cotton-wool, and autoclaved for 1 hr. at $1\frac{1}{2}$ atm. A sterile ascospore suspension was obtained by allowing moistened ripe perithecia to eject on to a sterile coverslip. One platinum wire loopful of the sterile suspension was added to each tube of sterilized soil. A sterilized, presoaked wheat grain was then dropped into each tube, and covered with moist sterile sand. Four of the twenty-four tubes were, however, deliberately recontaminated by addition of a trace of unsterilized soil to the ascospore suspension. For a comparison in unsterilized soil, twelve similar tubes, and twelve larger tubes, of size 2·8 × 20 cm. (to permit of longer growth of the wheat seedlings), were filled with the same weight, 28 g., of air-dry Bridgham soil. The soil of each tube was brought to a moisture content of 50% saturation by the addition of 5 c.c. of an ascospore suspension containing 24,000 ascospores per c.c., making 120,000 ascospores per tube. Germination of the ascospores on 0·5% dextrose agar was estimated at over 90%. Two wheat seeds were planted per tube and covered with moist sand. All tubes were kept in a north window at laboratory temperature, which fluctuated between 15 and 20° C. during the period of the experiment.

After one month, the wheat seedlings were dead in seventeen of the twenty tubes of sterilized soil inoculated with a sterile ascospore suspension and kept sterile. Examination of the roots showed severe infection and stunting by *Ophiobolus*, which had established itself in pure culture on the sterilized soil in these seventeen tubes. In the three tubes in which the seedlings remained alive, the roots of one seedling showed infection, but those of the other two remained healthy. Microscopical examination of all tubes before washing out had previously shown that whereas *Ophiobolus* was growing in pure culture throughout the soil of the seventeen tubes with dead seedlings, it had not succeeded in establishing itself throughout the soil of the other three tubes. This failure was attributed to chance contamination of the sterilized soil by other micro-organisms; in accordance with expectation, the seedlings in the four tubes deliberately recontaminated were found to be alive and healthy. In the twenty-four tubes of unsterilized soil, each inoculated with some 120,000 ascospores, all wheat seedlings were alive and apparently healthy; seedlings were washed out of the twelve smaller tubes at the end of the first month, but those in the larger tubes were left for a period of two months before root examination was made. Microscopical examination failed to reveal root infection in any of the forty-eight plants from the unsterilized soil.

This experiment was repeated, with essentially similar results. It then became necessary to determine whether ascospore infection could

take place in a sterile sand medium, with no other nutrients present than those deriving from the roots of the wheat seedlings. Since sterile sand offers no substrate for the multiplication of *Ophiobolus* mycelium, a large quantity of ascospores in bacteriologically sterile suspension was required for this experiment; it was obtained from perithecia which had formed in some cultures of *Ophiobolus* on oatmeal agar. The experiment comprised eighteen large test tubes (2·0 × 17·5 cm.), six with sterilized sand plus 1% glucose solution, six with sterilized sand without nutrient, and six with unsterilized sand without nutrient. Two sterilized and presoaked wheat seeds were planted per tube, and each tube received 1 c.c. of a bacteriologically sterile ascospore suspension containing some 14,000 ascospores. The two series of tubes with sterilized sand were planted and inoculated with full aseptic precautions. All tubes were then incubated in a glass jar kept in a north window of the laboratory (at 16–20° C.). After 14 days, severe root infection in both the series of sterile sand could be seen through the glass walls of the tubes; seedlings were washed out after 19 days. In both series of plants grown in sterile sand, the roots were discoloured brown throughout, and had been killed off short by the infection; stem lesions were also present on every plant. No infection by *Ophiobolus* could be found in the roots of the plants grown in the unsterilized sand. The striking difference in appearance between the severely infected plants from the sterilized sand (without glucose) and the healthy plants from the unsterilized sand is shown in Pl. III. Infection was perhaps slightly more severe in the sterilized sand with glucose; the effect of glucose would probably have been more apparent had a lower concentration of ascospores been used for inoculation.

These experimental results are thus in agreement with those of Kirby (1925), who obtained infection by ascospores of sterile wheat seedlings growing in pure culture in tubes or Petri dishes on 0·2% dextrose and potato agars. Mangin (1899) has figured infection of a wheat root hair by the germ tube of an ascospore. No other circumstantial accounts of ascospore infection have been found, though it seems to have been generally assumed that ascospore infection could occur, even if it were not of much importance in the dispersal or survival of the fungus.

III. Discussion

The occurrence of ascospore infection in sterile sand indicates that the nutrients provided by sterilized soil are unnecessary for the initiation of infection, and that sterile conditions alone are sufficient. It appears,

therefore, that the antagonism of other micro-organisms in some way prevents initiation of ascospore infection in unsterilized soils and even in unsterilized sand. Infection by agar inoculum disks of *Ophiobolus*, on the other hand, occurs freely in sand and in a variety of soils, and seems to be limited rather by physico-chemical conditions than by the antagonism of the soil microflora (Garrett, 1936). It is possible, however, that initial infection of a root by *Ophiobolus* can only occur at a certain nutritional level of the penetrating hyphae. The use of agar inoculum disks or of pieces of infected straw secures this nutritional level. In wheat seedlings grown under pure culture conditions in sterile sand, the excretion of organic material from the growing roots may provide the accessary nutrient necessary for successful ascospore infection. Work on root excretion and related phenomena has been reviewed recently by Loehwing (1937). In unsterilized soil and sand, however, these organic excretions and detrital material are rapidly assimilated by the general soil microflora. This aspect of the association of roots and soil micro-organisms has been investigated exhaustively by Starkey (1929–38), who has demonstrated a remarkable increase in numbers and activity of soil micro-organisms in the immediate vicinity of plant roots.

The observation that unsterilized soil and sand inhibits infection by ascospores but not by agar inoculum suggests that the inhibiting effect is connected in some way with the nutrition of the infection hyphae. Under sterile conditions, root excretions are wholly available to the ascospores and may make good the nutritional deficiency; in unsterilized soil, the root excretions are rapidly assimilated by the general soil microflora.

The relation of nutrition to germination and infection of the host by fungus spores has been reviewed recently by Brown (1936); it is becoming apparent that infection by some of the root-parasitic fungi is conditioned similarly by the nutritional level of the inoculum (Garrett, 1938*b*). In general, the greater the resistance offered by the host root to invasion, the higher must be the nutritional level of the fungus inoculum for successful penetration to occur. Thus, direct infection of the uninjured cork-covered older roots of forest and plantation trees by fungus spores has never been demonstrated and, in recent years, the importance of a "food base" behind the invading hyphae has been emphasized increasingly by Gadd (1936) and others.

In conclusion, the bearing of these results on the field occurrence of the take-all disease calls for some comment. It scarcely now seems likely that the ascospores can play any part in the propagation and dispersal

of the disease in the field, so that greater emphasis is laid on the underground survival and spread of the fungus, which is present probably to some extent in the majority of wheat-growing soils over its geographical range. The "sudden appearance" of the disease in a field where it has not been seen for some years is, therefore, to be attributed to changes in season or in farming practice, e.g. short rotations, which have favoured the fungus. This conclusion is supported by field observations made on the occurrence of the disease in the southern and eastern counties of England during the past few seasons (Garrett, 1938*a*). Severely diseased and clean crops were, not infrequently, seen standing side by side on the same land: such crops were sometimes on the two halves of one field on which different rotations had been followed. The extension of the disease to fresh areas may, therefore, be a slower process than has appeared hitherto; in order to explain it we seem to be thrown back once more on the original suggestion of McAlpine (1902) that dispersal is brought about by means of wind-blown fragments of infected plant material.

IV. Summary

Attempts to produce infection of wheat seedlings by the ascospores of *Ophiobolus graminis* in a variety of natural soils and in sand have failed. Yet, the ascospores germinate well on nutrient agars, and the resulting agar cultures produce infection as readily as cultures obtained from mycelium.

Ascospore infection of sterile wheat seedlings growing in sterilized soil may be obtained without difficulty. The nutrients present in sterilized soil are unnecessary for the initiation of ascospore infection, which occurs as freely as in sterile sand. Ascospore infection is, therefore, considered to be inhibited in unsterilized soils and sand by the antagonism or competition of other soil micro-organisms.

In unsterilized sand this antagonism is not sufficient to hinder infection by agar inoculum, nor does it appreciably impede the progress of infection along the roots. It is suggested, therefore, that microbiological interference with the initiation of ascospore infection is a competitive rather than an antagonistic effect, and is due to assimilation by the general soil microflora of the nutritive substances excreted from the growing and developing roots. Only under sterile conditions is this organic detritus available to the germinating ascospores.

The results of these experiments render it unlikely that the ascospores can play any part in the survival and dispersal of the fungus under field conditions.

GARRETT.—Soil Conditions and the Take-all Disease of Wheat (pp. 47–55)

I am much indebted to Mr Geoffrey Samuel for criticism of the experiments.

REFERENCES

ASTHANA, R. P. & HAWKER, L. E. (1936). The influence of certain fungi on the sporulation of *Melanospora destruens* and of some other Ascomycetes. *Ann. Bot., Lond.*, **50**, 325.

BROWN, W. (1922). Studies in the physiology of parasitism. VIII. *Ann. Bot., Lond.*, **36**, 101.

—— (1936). The physiology of host-parasite relations. *Bot. Rev.* **2**, 236.

GADD, C. H. (1936). Diseases of the tea bush. II. Root diseases. *Tea Quart.* **9**, 5.

GARRETT, S. D. (1934). Factors affecting the severity of take-all. I, II and III. *J. Agric. S. Aust.* **37**, 664, 799 and 976.

—— (1936). Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667.

—— (1938*a*). The take-all or whiteheads disease of wheat and barley, and its control. *J.R. agric. Soc.* **98**, 24.

—— (1938*b*). Soil conditions and the root-infecting fungi. *Biol. Rev.* **13**, 159.

HAWKER, L. E. (1936). The effect of certain accessary growth substances on the sporulation of *Melanospora destruens* and of some other fungi. *Ann. Bot., Lond.*, **50**, 699.

KIRBY, R. S. (1925). The take-all disease of cereals and grasses caused by the fungus *Ophiobolus cariceti*. *Mem. Cornell agric. Exp. Sta.* no. 88.

LOEHWING, W. F. (1937). Root interactions of plants. *Bot. Rev.* **3**, 195.

MCALPINE, D. (1902). Take-all in wheat. *J. Dep. Agric. Vict.* **1**, 74.

MANGIN, L. (1899). Sur le piétin ou maladie du pied du blé. *Bull. Soc. mycol. Fr.* **15**, 210.

SAMUEL, G. (1937). Whiteheads or take-all in wheat. *J. Minist. Agric.* **44**, 231.

SAMUEL, G. & GARRETT, S. D. (1933). Ascospore discharge in *Ophiobolus graminis*, and its probable relation to the development of whiteheads in wheat. *Phytopathology*, **23**, 721.

STARKEY, R. L. (1929–38). Some influences of the development of higher plants upon the micro-organisms in the soil. *Soil Sci.* **27**, 319, 355 and 433; **32**, 367 and 395; **45**, 207.

EXPLANATION OF PLATE III

Wheat seedlings inoculated with ascospores of *Ophiobolus graminis*: (*a*) in unsterilized sand, (*b*) under sterile conditions in sterilized sand.

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STUDIES ON *PUCCINIA ANOMALA* ROST.
I. PHYSIOLOGIC RACES ON CULTIVATED BARLEYS

By BRANQUINHO d'OLIVEIRA, Ph.D.[1]
Botany School, Cambridge

CONTENTS

Introduction

Comparatively little is known of physiologic specialization in *Puccinia anomala* Rost. The first account of experiments on the relative resistance of agronomic varieties of barley to *P. anomala* was published by Vavilov (1919). His results on eighty-two barleys showed the existence of resistant varieties, but it is not possible to compare his results with the reactions of existing physiologic races because there is no certainty that he was working with a pure inoculum from one physiologic race only.

Mains (1926) first drew attention to different physiologic races in *P. anomala* in the following statement: "Two physiologic forms of the leaf rust of barley have been distinguished by differences in reaction of the lines Oderbrucker C.I. no. 940, Featherston C.I. no. 1120 and Horsford C.I. no. 5057, all of which show high resistance to physiologic form 1 of *P. anomala* and are more or less susceptible to physiologic form 2."

Waterhouse (1927) published the reactions of 116 barleys inoculated with one stock culture of Australian rust. The barleys were from the species *Hordeum vulgare* L., *H. intermedium* Kake, *H. distichon* L., *H. deficiens* Steud., and also two wild barleys, *H. spontanenum* Kock and

[1] Now at Estação Agronomica Nacional, Edificio dos Jeronimos, Belem, Lisboa, Portugal.

H. murinum L. He stated that only seventeen barleys were resistant to his strain of *P. anomala*, fifteen belonging to *Hordeum vulgare* L., one to *H. distichon* L., and the other was *H. murinum* L. All the remaining barleys were more or less susceptible. Waterhouse (1929), in a new account of his experiments, stated that some of the varieties, which were resistant during the winter, were more or less susceptible in summer. Also, he emphasized that, in his new experiments, *H. murinum* was susceptible to *P. anomala*. This is an important point since no one else has recorded infection on this barley, and hitherto it has been considered immune. It seems probable that the new culture of Waterhouse was a physiologic race not yet studied.

Mains (1930) summarized his work on this rust. He used cultivated and wild barleys and also several grasses and gave the reactions of some of his agronomic varieties of barley to his two physiologic races of *P. anomala*. Also, he tested these races on twenty-six of the most resistant of the barleys used by Waterhouse.

Hirchhorn (1933), in Argentina, on the basis of field observations, reached the conclusion that *P. anomala* in La Plata exists in physiologic races different from those of Mains and Waterhouse.

Mains & Martini (1932) published a new account of the reactions of Mains's two physiologic races on about 600 varieties and selections of barleys in the greenhouse and in the field.

Brown (1931) described physiologic races of *P. anomala* found in Canada in 1929. These races were distinguished from one another on six new barleys, all different from Mains's differential hosts. Brown claimed that four physiologic races were differentiated in this way.

The most valuable selection of barleys was made by Hey (1932) from 273 barleys of the species *Hordeum polystichum* and *H. distichum*, from which he selected ten differential hosts. With these he was able to distinguish eight physiologic races in Germany and Bulgaria.

Again, in Germany, Ronsdorf (1934), working with Hey's barleys and with a new variety Aegyptische 4-zeilige (selected by Gassner & Straib, 1932), was able to identify two of Hey's races, numbers III and IV, and also a new race, which she numbered IX. Ronsdorf (1935) described two new races from America, X and XI, and also published the reactions of races II, III, IV, V and IX on some of Mains's differential barleys.

Stakman *et al.* (1935) referred to Hey's (1932) and Rondsdorf's (1934) results and modified the list of barleys selected by Hey. A new barley was added (Breuns Neuzucht 25) which had been tested but not selected by Hey. Also one of Hey's selected barleys was omitted.

Material and methods

(1) *Sources of cultures of* P. anomala *Rost.*

Seventy-seven collections of *P. anomala* from cultivated barleys, obtained from England, Portugal and Spain, were tested between February 1933 and June 1936.

All the remaining cultures were obtained from aecidia produced in 1936.

(2) *Establishment of cultures*

Whenever possible single-spore cultures were prepared from field material as soon as it was received. When the material arrived in bad condition or during a busy time a mass inoculation was made and single-spore cultures were established later.

(*a*) *Mass inoculations.*

A pot of eight to ten seedlings of Spratt Archer barley was used. Each plant in the pot was inoculated with spores taken from a single pustule chosen at random from the most isolated pustules on the leaf.

Incubation took place under bell-jars in which the atmosphere was kept damp. Sometimes during summer in Portugal it was necessary to line one-half of the bell-jars with damp blotting paper, and when the temperature rose above 26° C. the air was cooled with ice.

After 48 hr. incubation in the moist chamber the pots were transferred to benches in the greenhouse and the plants were kept under lamp glasses. The top of each lamp glass was covered with a layer of cotton-wool over which a small square of muslin was placed, the whole being held by an elastic band. If spore-proof cellophane cylinders were available these were used instead of the lamp glasses, since they allowed the air inside to be kept fairly dry.

(*b*) *Establishment of single-spore cultures.*

To establish single-spore cultures three methods were tried, the agar method, Newton & Johnson's method (1932), and also a new method consisting of transfer of the spores from a dry glass slide with a wet glass capillary needle. The capillary tube was dipped in sterile water and used to pick up individual spores from the slide. The spores were transferred to the leaf by blowing gently through the mouthpiece of the pipette. The pipettes were sterilized between inoculations. The barley seedlings were then kept at room temperature until the droplets of water evaporated; this was done to prevent the drops from running down the leaf and mixing the spores. Incubation was allowed for 48 hr. as described by Newton & Johnson (1932), after which the pots were covered with cellophane cylinders. With this method the average number of infections obtained was 20–50%. As soon as the flecks of pustules were visible only one was allowed to develop on each leaf. The remaining flecks were covered with a layer of vaseline on both sides of the leaf. Each leaf was then numbered and covered with a test-tube kept in position by a zinc support. Three days after the pustules had made their appearance, uredospores were taken from each pustule to a new pot of barley seedlings in order to establish stock cultures.

(3) *Maintenance and preservation of stock cultures*

All stock cultures were maintained on seedlings of Spratt Archer barley kept under the cellophane spore-proof cages or in the rust-free room of the greenhouse. Inoculations were made on the first leaf of the seedlings when the second leaf began to appear.

The inoculum was collected from stock cultures by means of a sterilized scalpel. A small amount of inoculum was applied to the upper surface of the leaf and spread on it with a sterile brush.

After incubation the cultures were kept free from contamination by air-borne spores. Lamp glasses could only be used in dry weather; during wet weather the rust pustules became mouldy.[1] The cultures kept longer and better if the spore-proof cylinders were made with a special watering place in order to avoid the necessity of lifting the cylinder to water the pot. The cylinder had to be forced deeply into the soil and, during dry weather, it was necessary to see that the cellophane did not become torn. In order to prevent contamination of the cylinders by spores of *Erysiphe* and saprophytes the covers were autoclaved every time new inoculations were made. Under Portuguese conditions, particularly in Lisbon, care was necessary to keep out ants. Stock cultures were renewed every 3 weeks.

When the English physiologic races and some of the Spanish physiologic races had to be transported from England to Portugal in December 1934 they were taken on Spratt Archer seedlings cultivated in Knop's agar in test-tubes by the technique of Ward (1902) and Mains (1917). Cultures were also stored in a refrigerator in a desiccator in which the relative humidity was 50% as advised by Peltier (1925) and Newton & Johnson (1932).

(4) *Differentiation of the physiologic races*

(*a*) *Differential hosts.*

Differentiation of physiologic races of *P. anomala* was made chiefly on the basis of Hey's selection of barleys (1932).

Aegyptische 4-zeilige Sommergerste, selected by Gassner & Straib, and used by Ronsdorf (1934), was also tested.

Seven of Mains's differential barleys have also been used.

The following is the list of all barley varieties used as differential hosts:

Hey:	*Hordeum hexastichum eurylepis*
	Hordeum hexastichum recens
	Hordeum vulgare speciale
	Breustedts Schladener
	Friedrichswerther Berg Wintergerste
	Australische Recka
	Samaria 4-zeilige
	Hordeum vulgare pallidum (Sudan)
	Lichtis Lechtaler
	Ackermanns Bavaria
Gassner & Straib:	Aegyptische 4-zeilige Sommergerste
Mains:	Featherston C.I. no. 1120
	Oderbrucker C.I. no. 940
	Malting C.I. no. 1129
	Hanna C.I. no. 906
	Quinn C.I. no. 1024
	Bolivia C.I. no. 1257
	Juliaca C.I. no. 1114

[1] *Hyalopus* sp. (*H. parasitans* B. & C. (?)) was the most common mould on the cultures. *Cephalothecium roseum* Corda and *Fusarium* sp. were also recorded.

Seeds of these barleys were kindly supplied to Prof. F. T. Brooks by Dr Hey and Dr A. B. Mains.

The first experiments were carried out with the original seed, but later seeds from crops grown at Cambridge and Lisbon were used.

With one exception all the barleys reacted more or less as pure lines to our physiologic races. Later investigations showed that Friedrichswerther Berg Wintergerste had a small amount of foreign seed mixed with it which proved to be *Hordeum distichum*.

(*b*) *Infection types and reaction classes.*

The classification of the reaction classes adopted in the course of this work is the one described by Mains & Jackson (1926) and subsequently used by Waterhouse (1927), Mains (1930), Mains & Martini (1932), Hey (1932) and Ronsdorf (1934, 1935):

Type 0 = *Extremely resistant.* No uredo pustules. Infection only visible as small points or flecks, sometimes in rings, but chlorotic areas do not become darkened.

Type 1 = *Very resistant.* Uredo pustules minute, few. Infection chiefly visible as necrotic flecks without pustules.

Type 2 = *Moderately resistant.* Uredo pustules rather small. Chlorotic and necrotic flecks predominating, usually without pustules.

Type 3 = *Moderately susceptible.* Uredo pustules of moderate size or large, more or less numerous. Generally some chlorosis.

Type 4 = *Extremely susceptible.* Uredo pustules large, numerous and generally confluent. True hypersensitiveness absent, but a little chlorosis when unfavourable conditions exist.

Type X, as described by Stakman & Levine (1922) for *P. graminis tritici*, was added to Mains's symbols because it seemed to be the only means of representing the rather heterogeneous reaction often shown on Bolivia and Juliaca barleys:

Type X = *Heterogeneous reaction.* Uredo pustules very variable, apparently including all types and degrees of infection often on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones and vice versa.

"Variable" or "intermediate" types of reaction such as 0+1−, 1+2−, 2+3−, 3+4−, 1·2, 2·3, etc., and 1+ or 1−, 2+ or 2−, etc., were also used when the type of reaction was not sharply defined. However, on final readings a fixed type was allotted whenever possible.

(*c*) *Inoculation and incubation.*

Testing of pure stock cultures was carried out on seedlings growing in good loam soil in small pots.

The whole range of Hey's and Mains's barleys was usually tested at the same time with any one culture. When possible more than one culture (often as many as five) were tested on the same day in order to make the comparison of the reactions of different cultures as easy as possible. The method of inoculation was the same as for the maintenance of the stock cultures. Care was taken to avoid heavy spore inoculation, which in some susceptible varieties, as pointed out by Hey (1932), causes abnormal necrotic zones. The incubation of the whole set of differential hosts, inoculated with the same

culture, took place under a small glass frame for 48 hr. After incubation the pots were transferred to the benches in the greenhouse and were adequately watered.

In winter, under Portuguese conditions of light and at a mean temperature of 10–14° C. (Lisbon), flecks were visible in 4 days in susceptible varieties and pustules were open on the eighth or ninth day after inoculation. Under English winter conditions at Cambridge with very low light intensity and in the cold compartment of the greenhouse (mean temp. 5·3° C.), the flecks were sometimes visible only on the sixth or seventh day and pustules were open about a week later. Under the same conditions of light but in the warmer house (mean temp. 14·8° C.) pustules were out on the ninth or tenth day. With high light intensity and mean temperature of 15–19° C. flecks were visible about 3–4 days after inoculation and pustules were coming out on the eighth or ninth day. During summer, when the temperature did not exceed 22–26° C., pustules were often open on susceptible varieties within 6 days. When the weather was very hot—27° C. or over—the pustules sometimes began to appear on the sixth day, but the reaction was not fixed until some time later. Generally speaking these results are very similar to those obtained by Hey.

Determinations of the reaction type were made three times: the first observation was made when pustules appeared, the second 3 days later, and the third within 2 or 3 days to confirm the previous observations. As a rule the first two readings are adequate.

(*d*) *Method of determining new physiologic races and frequency of tests.*

Every single-spore culture was tested on the differential barleys as soon as possible after isolation. When the reactions of the first set of inoculations coincided exactly with those of any of the physiologic races already studied the culture was discarded.

On the other hand, if the reactions of a particular culture did not agree closely with those of known physiologic races a new test was carried out on the whole range of hosts side by side with tests of those cultures closely related to it. Care was then taken to keep the sets under exactly the same conditions, and the readings of the reactions were taken at the same time. If the new culture could not be identified with any of the others it was kept in order to determine the reaction variations. The cultures could be tested practically throughout the year. In Cambridge, however, during dull weather from November to February the reactions sometimes became confused; in Portugal, during very hot summer months, if the barleys were not kept under the cooled frames for at least 48 hr. after inoculation, infection was very irregular and often only a few pustules could be observed. Such results were ignored.

The number of times the individual tests were made varied somewhat. The early cultures have been tested since the end of 1933, while the more recent ones, some of which were isolated during the spring and summer of 1936, have been tested only twice. No account is given of any physiologic race which was not tested at least twice on all Hey's and Mains's barleys.

EXPERIMENTAL RESULTS

(1) *Reactions of the physiologic races isolated*

From the eighty-two cultures of *P. anomala* established from uredosporic material, eleven new physiologic races were isolated. The extreme reactions of each of these new races are summarized in Table I, and the

provisional numbers of 12 to 22 are ascribed to them. None of the races previously described by Hey (1932), Ronsdorf (1934, 1935) and Mains (1930) was found.

One of the races, 12, was found to be widely distributed in England, Portugal and Spain. The remaining ten were localized. Races 13, 14, 15, and probably 18,[1] were found on English material, races 18, 19 and 20 on Portuguese material, and races 16, 17, 21 and 22 on Spanish material.

(2) *Differences between physiologic races*

Two of the new races, 16 and 17, resemble race 1 closely as regards their reactions on Hey's differential hosts. As the reactions of this last race on Mains's and Aegyptische 4-zeilige Sommergerste barleys are not known, distinction between race 1 and races 16 and 17 was only possible on Samaria 4-zeilige. The differences on this barley are slight, the reactions being 3 – 2 for race 1 and 3 + 4 –, 4 for races 16 and 17. However, differences as small as these have already been adopted by Hey to differentiate races 4 and 5. Further evidence to maintain them as separate races is based on the reactions on Quinn, which is immune to race 17 (–) and only resistant or moderately resistant (1, 3 –) to race 16.

Race 11, isolated by Ronsdorf from North American material, Michigan, and tested only on Hey's barleys, is somewhat similar to race 12, but is easily differentiated on *Hordeum vulgare speciale* and on Breustedts Schladener, both with a reaction type 3 – 2 for race 11 and 4 –, 4 for race 12.

Race 13 can be differentiated from race 12 and from race 11 by the reactions on Australische Recka (3 + 4 –, 4 for race 12, 1, 2 for race 13 and 3 for race 11). Race 8, tested only on Hey's barleys, showed reactions very similar to those of race 20. Differentiation was established chiefly by the reactions on Samaria 4-zeilige (2–3 for race 8, 4 for race 20). Lichtis Lechtaler and Ackermanns Bavaria are also somewhat more susceptible to race 20 (reaction 2) than to race 8 (reaction 1).

Races 18, 19, 20, 21 and 22 are more sharply distinct from the races already described by Hey and Ronsdorf. They form a group of which the main feature is high resistance shown by Hey's barleys, *H. vulgare speciale* and Breustedts Schladener (reaction 0 to 1 +), and by Mains's barleys, Featherston, Oderbrucker and Malting (reaction 0 to 2).

Race 19 is clearly differentiated from the other four on Friedrichswerther Berg Wintergerste, which is highly susceptible to this race

[1] A culture established from material sent from Reading and only tested once reacted apparently as race 18. The culture was lost soon after the first test.

Table I. *Extreme reactions of eleven new physiologic races established from uredosporic material of* P. anomala

Year ...	1934–6	1934–6	1934–6	1934–6	1934–6	1934–6	1935–6	1936	1936	1936	1936
Physiologic races ...	12	13	14	15	16	17	18	19	20	21	22
Country ...	England Portugal and Spain	England	England	England	Spain	Spain	Portugal	Portugal	Portugal	Portugal and Spain	Spain
Barleys:											
Aegyptische 4-zeilige Sommergerste	3+4–, 4	3+4–, 4	2, 3+	3, 4	0, 2–	0, 1+2–	4	4	4	2–, 3	4 , 4
Hordeum hexastichum eurylepis	4	4	4	3, 4	4	4	4	3+4–	4–	4–	4
Hordeum hexastichum recens	4	4	3+4–, 4	3+4–, 4	4	4	4	4	4–	3, 3+	4
Hordeum vulgare speciale	4	4	1+, 4X	0, 1	0, 2	0, 2	0, 1	0, 1	0	0	0, 1
Breustedts Schladener	4–, 4	4	1, 4–	0, 1	0, 2	0, 2	0, 1	0, 1	1	0	1+
Friedrichswerther Berg Wintergerste	3+4–, 4	3+4 , 4	2, 4 , X	0, 1-2*	0, 2	0, 2	0, 1	4	1	0	1+
Australische Recka	3+, 4	1, 2	2, 4	3, 4	0, 2	0+, 2	4	4	4 , 4	3-4*	4
Samaria 4-zeilige	3+4 , 4	4	4–, 4	3+4 , 4	4	4	4	4	4	4	2 , 2
Hordeum vulgare pallidum (Sudan)	4	4	3–, 3	4	4	4	4	4	4	4-	4
Lichtis Lechtaler	3 , 4–	3, 4	3+4–, 4	4	3+4- , 4	3+4 , 4	4	2+3–	2	4–	4
Ackermanns Bavaria	3–, 4	3, 4	3, 3+4–	3+4 , 4	3+4 , 4	3+4–, 4	4	2	2	4–	4
Featherston C.I. no. 1120	4	4	X, 2, 3–4	1, 2+3	1+2	0, 2	0, 1+	1+, 2	2	0	1+2–
Oderbrucker C.I. no. 940	4–, 4	4	1, 3+	0, 1-2*	0, 2	0, 2	0, 1+	1	2–	0	1, 2
Malting C.I. no. 1129	4–, 4	4	1+2–, 3+	0, 1	0, 2	0, 2	0, 1+	1	1+	0	1, 2
Hanna C.I. no. 906	4	4	4–, 4	3+4–, 4	4	4	4	4	4	4	4
Quinn C.I. no. 1024	3, 4	3, 4	2, 3+4–	1, 2+3–	2, 3	0	4	4	4	3+4–	1–, 2–
Bolivia C.I. no. 1257	4–, 4X, 4	4X, 4	3+4–, 4X	3, 4, X	3X, X	3, X	3+4–	4	3	2, 3	4
Juliaca C.I. no. 1114	4–, 4X, 4	4X, 4	3+4–, 4X	X, 4–, 4	X	X, 4	4	4	4–	3	X, 4

* =reaction range of 1 to 2 or 3 to 4.

(reaction 4), while it proves to be highly resistant to races 18, 20, 21 and 22 (reaction 0 to 1+). Race 22 is also differentiable from the other three on Quinn, which is resistant to this race (reactions 1− and 2−) and is susceptible to the other races, with reaction types 3+4− and 4.

The remaining three races (18, 19 and 21) are still more closely related; their distinction, however, is possible on Aegyptische 4-zeilige which is moderately resistant (2− to 3−) to race 21 and susceptible to races 18 and 20. Finally, these last two can be differentiated on Lichtis Lechtaler, susceptible to race 18 (reaction 4) and moderately resistant (reaction 2) to race 20.

Race 14 was rather inconstant as regards its reactions. There was always a great deal of variation on several of the most important differential barleys. Reaction types with this race varied as much as from 1+ to 4 and X on *H. vulgare speciale*, 1 to 4− on Breustedts Schladener, and 2 to 4− and X on Friedrichswerther Berg.

(3) *Mutation in physiologic race* 14

Colour mutation in *P. anomala* was noticed in June 1934. During a test of race 14 on the standard barleys, several orange pustules were observed amongst brown ones on *Hordeum vulgare pallidum* (Sudan). The orange pustules were not attributed to mutation at the time. In July, when new tests were being carried out with the same race, more orange pustules were observed on the same barley. Single-spore isolations from these orange pustules on *H. vulgare pallidum* were then made on Spratt Archer, and from these stock cultures were obtained. These cultures maintained the orange colour although it was not quite as bright as on the original barley. In August a comparative inoculation test was made on the differential hosts. It was then seen that the colour mutation was accompanied by mutation in pathogenicity, for on some of the barleys the reactions were different from those of race 14 (see Table II). This mutant has been numbered physiologic race 23.

During these preliminary experiments in July and August, light orange pustules appeared several times in other tests with physiologic race 14 on *H. vulgare pallidum* amongst the normal brown ones. From one of these brown pustules on *H. vulgare pallidum* a new single-spore culture was established and maintained on this barley in order to find out whether mutation would occur in a culture originating from a spore which had kept normal under conditions that induced mutation in others. In a later transfer several orange pustules again appeared. From September 1934 until May 1935 no more light-coloured pustules appeared in any of

the cultures of race 14. Then in May and June 1935, when new tests were being made with this race on *H. vulgare pallidum* in Portugal, under spore-proof conditions, further orange and a few light yellow pustules appeared. New single spore cultures were established from the yellow ones.

Table II. *Reactions of parent race* (*no.* 14) *and its mutant derivative* (*no.* 23)

	Extreme readings		Comparative test: Parent race 14		Comparative test: Mutant race 23	
Barleys	Parent	Mutant	25. ix	20. xi	25. ix	20. xi
Aegyptische 4-zeilige Sommergerste	2, 3+	2−, 3, 3X	3	2	3	2−
Hordeum hexastichum eurylepis	4	3+, 4−, 4	4	4	4	3+
Hordeum hexastichum recens	3+4−, 4	4	4	4−	4	4
Hordeum vulgare speciale	1+, 4X	0, 1−	3	4X	0·1−	0
Breustedts Schladener	1, 4−	0, 1	4−	X	0	0
Friedrichswerther Berg Wintergerste	2, 4−, X	0, 1	4	X	0	0
Australische Recka	2, 4	4−, 4	2	4	4	4
Samaria 4-zeilige	4−, 4	4	4	4	4	4
Hordeum vulgare pallidum (Sudan)	3−, 3	4	3	2	4	4
Lichtis Lechtaler	3+4−, 4	3+4−, 4	3+4−	3+4−	4	3+4−
Ackermanns Bavaria	3, 3+4−	4	3+4−	3	4	4
Featherston C.I. no. 1120	2, 3+4−X	0	3+4−	X	0	0
Oderbrucker C.I. no. 940	1, 3+	0, 0·1	1	3+	0	0
Malting C.I. no. 1129	1+2−, 3+	0	1+2−	3+	0	0
Hanna C.I. no. 906	4−, 4	4	4	4	4	4
Quinn C.I. no. 1024	2, 3+4−	2+, 4	2+	3−	3−	3+4−
Bolivia C.I. no. 1257	3+4−, 4, X	0, 2	3+4−	3+4−	1+	0
Juliaca C.I. no. 1114	3+4−, 4, 4X	4, 4X	3+4−	3+4−	4	4

A new comparative test between race 14, the first orange mutant (race 23), and the light yellow culture was then carried out. The light yellow mutant proved to be very similar to, if not identical with, the orange mutant as regards pathogenicity and was therefore classed as physiologic race 23.

The orange and yellow mutants appeared several times until about the middle of October 1935. After that, although some light-coloured pustules were observed, they were not stable, for when subcultures were made brown pustules always appeared.

It seemed that temperature probably played an important part in the occurrence of these changes. In order to test this an experiment was set up early in 1936 in which seedlings of *H. vulgare pallidum*, growing in pots, were inoculated on the same day with uredospores from culture 14. After an incubation period of 24 hr. the pots were divided into two sets. One set was left on the bench of the greenhouse where the temperature

range was 15–25° C. maximum to 8·5–14·5° C. minimum. The other set was transferred to an incubator with a glass door at the top, the temperature being maintained at 25–27° C. The seedlings in the greenhouse never showed any light-coloured pustules, while on those kept in the incubator several orange pustules appeared among the brown ones. Isolations were made from these orange pustules, but reversion to the brown colour always occurred.

Occasionally some of the other physiologic races, namely, 15, 16, 17 and 18, produced a very few orange pustules on *H. vulgare pallidum* as well as on Bolivia and Juliaca. However, all attempts to obtain new colour mutants failed.

The above results seem to confirm Stakman's (1930) assertion that the "frequency of mutation can be influenced by environmental conditions, such as amount and kind of nutrients and temperature".

In the mutation of race 14 the parts played by the nature of the host and the temperature seem quite definite. As yet, it is not possible to determine whether temperature has a direct effect or whether it acts indirectly by producing changes in the host.

Another important factor to be considered is the degree of instability of the physiologic race itself.[1] In the mutations that have been observed under controlled conditions, it appears that this phenomenon of reaction instability plays an important part. All four mutations found by Stakman *et al.* (1930) resulted at different times from the same culture of race 1 of *P. graminis tritici*. The mutant of *P. glumarum tritici*, reported by Gassner & Straib (1932), occurred on thirty-four cultures of one strain of the rust. Cotter & Levine's (1932) colour mutant of *P. graminis secalis* was observed simultaneously in two cultures of the same origin. Roberts (1936) described a mutant of race 66 of *P. triticina* which arose from a culture apparently unstable in reaction. The culture of physiologic race 14 of *P. anomala*, from which the present mutant was produced, was very inconstant in reaction. Another interesting point is the constancy with which the mutation took place on one particular barley, *Hordeum vulgare pallidum* (Sudan). The influence of the host as a determinant in the occurrence of mutation in this culture seems so well marked that it may support another interpretation of Ward's (1903) "bridging host" theory. The "bridging host" might in fact induce persistent changes in pathogenicity.

[1] This instability may concern only some strains of one race, as Roberts (1936) found in the case of race 66 of *P. triticina*. It is necessary to keep in mind, as Johnson *et al.* (1934) emphasize, that different cultures of the same physiologic race may be genetically different.

(4) *New physiologic races obtained from aecidia*

Craigie (1927–31) carried out the pioneer work on the relation of the pycnidia to the aecidia. For several rusts new physiologic races have been produced by hybridization or self-fertilization, notably by Waterhouse (1929), Newton *et al.* (1930*a*, *b*, 1932) and Murphy (1935).

No records of similar investigations on *P. anomala* have hitherto been published. Early in 1936 I succeeded in producing aecidia on species of *Ornithogalum* with sporidia of *P. anomala*, as will be described in detail in another paper. From the aecidia new physiologic races were obtained by segregation and hybridization which will now be described.

(*a*) *Material and methods.*

The *Ornithogalum* plants, grown in pots, were inoculated with sporidia and were then kept under cheese cloth frames or cellophane cylinders. The technique of Craigie (1931) or of Newton & Johnson (1932) was used.

For purposes of hybridization, leaves with a single haploid infection were chosen. Usually not more than one infection per plant or per group of plants was selected. These infections were allowed to develop for not less than 3 weeks to make sure that no self-fertilization had taken place. Only then were these pustules set apart to be hybridized.

Crossing of two physiologic races was done by transferring spermatial nectar from one infection to another and vice versa with a sterile needle or brush. Unfortunately, material for hybridization, under the conditions described above, was not obtained at the same time for all the physiologic races with which infections on *Ornithogalum* were obtained.

On the other hand, in some of the physiologic races self-fertilization could easily be brought about by mixing spermatia from different pustules. All but one of the new physiologic races obtained through the aecidial stage were produced in this way.

(*b*) *Pure cultures from aecidia.*

Previous workers have shown that in the heterozygous races, distinct physiologic races can be obtained from different aecidia of a single infection; care was therefore taken to avoid mixing the spores of separate aecidia.

The first method attempted to establish pure cultures from aecidia was that described by Newton & Johnson (1932) for the aecidia of *P. graminis*. But, as *Aecidium Ornithogalum* does not form a cupulate body outside the leaf, it was almost impossible to detach the aecidia from the leaves by means of forceps. Even when that was successfully achieved and chains of aecidiospores obtained, these showed very poor germination if compared with the freshly ripened spores.

Mass inoculations from a single aecidium are not advisable with this rust because later experiments showed that more than one physiologic race could be isolated from the spores of a single aecidium. Accordingly, a new technique was devised and tried with encouraging results.

One aecidial pustule was chosen and all the aecidia which were already open were covered with a layer of vaseline and only a single unopened aecidium was left uncovered.

This was done to prevent any possibility of the spores becoming mixed. As soon as the peridium of the uncovered aecidium split the spores were picked up with a sterile needle and scattered on a sterile slide. The aecidium was then covered with vaseline to prevent its spores from becoming mixed with those of aecidia which developed subsequently. By this method it was possible to isolate spores from more than one aecidium in a single infection without danger of mixing them.

From the aecidiospores on the slides, single-spore inoculations were made on Spratt Archer seedlings by the usual technique. When pustules appeared on the barley pure cultures were established.

The physiologic races from which aecidia were obtained are nos. 12, 13 and 23. Abundant material was obtained from nos. 12 and 13, but with no. 23 only a few infections resulted.

Physiologic races obtained from each source are described separately.

(*aa*) *Aecidia from infections obtained with physiologic race* 12

Infection I. Single-spore cultures were attempted from twenty-one aecidia but from only fifteen of them could cultures be established. From the progeny of these fifteen aecidia six physiologic races were identified. Two proved to be physiologic races 12 and 13 respectively. Another was race 16, or a very similar one. The other three were new races, and these are described in Table III under the numbers 24, 25 and 26.

In thirteen cases out of the fifteen the cultures resulting from spores of the same aecidium behaved as the same race, but in the two other aecidia (nos. 4 and 21) more than one physiologic race was isolated. From aecidium no. 4 one culture could be identified with the mother culture (race 12) and two others with race 24. From aecidium no. 21 races 12 and 26 were differentiated. The frequency with which the races appeared in the progeny is as follows:

Physiologic races segregated by race 12:	12	24	13	25	16	26
No. of aecidia in which the race was found:	6	4	3	2	1	1

Infection II. From this infection eleven aecidia were selected and it was possible to establish cultures from nine of them. The cultures so obtained were found to belong to physiologic races 12, 19 and 24 in the proportion of 7 : 1 : 1.

Infection III. In this infection isolations from twenty-eight aecidia were attempted, but cultures were obtained from seven only. Four of the aecidia developed as race 12, one as race 24 and the other two as a new race which was numbered 27.

The heterozygous condition of physiologic race 12 is clearly revealed by the progeny of the thirty-one aecidia produced in three infections. In

this progeny eight different physiologic races were identified. Four of them proved to be already known, or at least they gave the same reactions on our range of hosts as four of those found in nature. These four were 12, 13, 16 (or a variation of 16) and 19. The other four races were new and they were numbered 24, 25, 26 and 27.

(*bb*) *Aecidia from infections obtained with physiologic race* 13

Infection I. Isolations were attempted from twenty-three aecidia and single-spore cultures were obtained from nineteen. These proved to belong to five physiologic races. Three reacted as races 12, 13 and 22; the other two proved to be new and were numbered 28 and 29. Their reactions are given in Table III.

Table III. *Reactions of physiologic races* 24–29

Physiologic races ...	24	25	26	27	28	29
Barleys:						
Aegyptische 4-zeilige Sommergerste	0	4	4, 4X	4–	4	4, X
Hordeum hexastichum eurylepis	4	4	4	4	4	4
Hordeum hexastichum recens	4	4	4	4	4	4
Hordeum vulgare speciale	4	4–, 4	0	4	4	4
Breustedts Schladener	4	0, 1	0	4–, 4	0, 1+	4
Friedrichswerther Berg Wintergerste	4	0, 1	0, 1–	4	0, 1	4
Australische Recka	4	4	4	0, 0-1	4	2+
Samaria 4-zeilige	4	4–, 4	4	4	4	4
Hordeum vulgare pallidum (Sudan)	4	4	4	4	4	2
Lichtis Lechtaler	3, 3+	3+4–	2–, 2+3–	2	3+4–	3+4–
Ackermanns Bavaria	3, 3+	3+4–	2–, 2+3–	2, 2+	3+4–	4–
Featherston C.I. no. 1120	4	4	1, 1+	4	4	4
Oderbrucker C.I. no. 940	4	4	1	4	4	4
Malting C.I. no. 1129	4	4	0, 1	4	4	4
Hanna C.I. no. 906	4	4	4	4	4	4
Quinn C.I. no. 1024	2+3–, 3	1-2, 2	2	2, 2+	4	1+2–
Bolivia C.I. no. 1257	4, X	4–, 4X	4	4	4	4
Juliaca C.I. no. 1114	4–, X	4, X	4	4	4	4
No. of aecidia in which the race was found:	6	2	1	2	1	3

Only one physiologic race was found in each aecidium. The proportion in which the races appeared is as follows:

Physiologic races segregated by race 13:	13	22	12	29	28
No. of aecidia in which the race was found:	9	4	3	2	1

Infection II. Cultures were obtained from ten aecidia, and physiologic races 13, 22 and 24 were identified in the proportion of 4 : 4 : 2.

(*cc*) *Aecidia from infections obtained with physiologic race* 23 (*yellow*)

Only one infection, fertilized with spermatial nectar of the same origin, was available for aecidiospore isolation. Eleven aecidia from this infection were selected and in their progeny four physiologic races were segregated, viz. nos. 12, 19, 20 and 22.

All the cultures from aecidiospores showed only brown uredospore pustules. Colour segregation, as described by Newton & Johnson (1932) in the progeny of a colour mutant of *P. graminis tritici*, was not detected in the limited number of isolations attempted.

(*dd*) *Results of a reciprocal cross between physiologic races* 13 *and* 23

Three crosses between haploid infections of race 13 and haploid infections of race 23 were attempted. From the two hybridizations so obtained cultures were made and tested in the usual way. When race 23 was fertilized with spermatia of 13 the aecidiospores which were isolated gave rise to a new physiologic race described in Table IV as no. 30. From the reciprocal cross physiologic race 30 was found in five aecidia and race 18 in two others.

Table IV. *Reactions of races* 13 *and* 23 *and of their reciprocal crosses*

Physiologic races ...	13→23	13←23		Crossed races	
	30	18	30	13	23
Barleys:					
Aegyptische 4-zeilige Sommergerste	0, 1 –	4, 4X	0, 0·1	3+4–, 4	2–, 3, 3X
Hordeum hexastichum eurylepis	4	4	4	4	3+4–
Hordeum hexastichum recens	4–, 4	4	4	4	4
Hordeum vulgare speciale	0	0, 1	0·1–, 1	4	0, 1 –
Breustedts Schladener	0	0, 0·1	0, 1 –	3+4–, 4	0, 1
Friedrichswerther Berg Wintergerste	0, 1	0, 1	0, 1 +	1, 2	0, 1
Australische Recka	3+4–, 4	4–, 4	4	4	4–, 4
Samaria 4-zeilige	4	4	4	4	4
Hordeum vulgare pallidum (Sudan)	4	4	4	4	4
Lichtis Lechtaler	4	4	3+4–, 4	3, 4 –	3+4–, 4
Ackermanns Bavaria	4	4	3+4–, 4	3, 4 –	4
Featherston C.I. no. 1120	1	1	1	4	0
Oderbrucker C.I. no. 940	0, 1	1	1	4	0, 0·1
Malting C.I. no. 1129	0, 1 +	1	0, 2 –	4	0
Hanna C.I. no. 906	4	4	4	4	4
Quinn C.I. no. 1024	1	4	0, 1 –	3, 4	2+, 4
Bolivia C.I. no. 1257	4, 4X	4, 4X	4, 4X	4, 4X	0, 2 –
Juliaca C.I. no. 1114	4, 4X	4 –	4, 4X	4, 4X	4, 4X
No. of aecidia in which the race was found:	6	2	5	—	—

Behaviour in this hybridization, in which one of the reciprocal crosses gave rise to two physiologic races, is somewhat different from the results

of Newton & Johnson (1932) with *P. graminis tritici*. These authors stated that in some reciprocal crosses cytoplasmic inheritance could be demonstrated by the origin of different physiologic races, but in no case was more than one race isolated from each infection. Goldschmidt (1928) postulated that for *Ustilago violacea* Pers. such genetic characters are cytoplasmic. On this basis the above results with *P. anomala* could only be explained by supposing that more than one hypha had been fertilized by different spermatia. If so, the cytoplasms might carry pathogenic factors capable of modifying the general type of pathogenicity dependent on the hybrid nucleus.

(5) *Biotypes of physiologic races* 12 *and* 13

During our experiments a large number of cultures, identified with races 12 and 13, showed slight but constant differences in reaction. These differences were observed chiefly on Australische Recka, Lichtis Lechtaler and Ackermanns Bavaria. The reactions of some of these cultures were compared under the same conditions.

Thus, from race 12 cultures 35, 54 and 71 were inoculated at the same time. They were incubated together and maintained on the same bench of the greenhouse. The following results were obtained:

	Cultures		
	54	35	71
Australische Recka	3, 3+	4	3+4−
Lichtis Lechtaler	4	3+4−	2+3−
Ackermanns Bavaria	4	3+4−	2+3−

Also ten single-spore cultures from an aecidium, which apparently reacted as race 13, were compared in the same way. These cultures were numbered "13, aec. 4, 1 to 10", but will be referred to as 1, ..., 10. It was not possible to inoculate all of them at the same time, but two sets of inoculations were made, using five cultures each time, and an interval of 2 days was allowed between the two series. During the time of the experiment there was no significant change of temperature to influence the reactions. The results confirm the preliminary observations, for in both sets of inoculated barleys sharp differences were observed. The reactions on the three barleys were as follows:

	Cultures									
	3	7	2	4	6	8	9	1	5	10
Australische Recka	0	0, 1	1	1	1+	2	2	2+	3−	3
Lichtis Lechtaler	3	4	2+	3	3	3−	2	3−	3+4−	3+
Ackermanns Bavaria	3	4	2+	3	3	3	2+	3−	3+4−	3+

It is difficult to decide whether some of these biotypes ought to be considered as belonging to races 12 or 13. According to the present definition of a physiologic race, culture no. 3 (reaction type 0) on Australische Recka is different from culture no. 1 (reaction type 2+) and is even more distinct from culture no. 10 (reaction type 3). However, the differences become less evident when the two extremes are compared with the original type culture, which gives a reaction 1, 2. Moreover, considering the reactions of races 12 and 13 on Australische Recka which are respectively 3+, 4 and 1, 2 and the reactions of the ten biotypes on the same barley, it is possible to construct a continuous series linking together the two races.

This hypothesis, suggested by the experiments, is corroborated by the existence of very closely related races of *P. graminis* and *P. triticina*. Stakman & Levine (1935) call attention to this in the "Analytical Key for the Identification of Physiologic Forms of *P. graminis tritici*": "It is well to keep in mind that in some cases what is designated as a form may in reality comprise several closely related biotypes. Furthermore, some forms (races) are so closely related that it is difficult to distinguish between them. It appears that there is an indefinite number of biotypes of *P. graminis tritici* many of which differ from each other almost imperceptibly."

A new arrangement of differential hosts for the physiologic races of *P. anomala*

The reaction tests of the nineteen new physiologic races described above were tried on Hey's differential set, on some of Mains's differential barleys and on Aegyptische 4-zeilige Sommergerste. The results showed that a new arrangement of the standard differential hosts selected by Hey was necessary to make it efficient.

In Hey's collection of barleys some hosts are duplicated at least as far as concerns the reactions of the races which are already known. On the other hand, it fails to show differences between those of our races which are differentiable on other hosts. Thus Lichtis Lechtaler and Ackermanns Bavaria both react in the same way to all the physiologic races. *Hordeum hexastichum curylepis* and *H. hexastichum recens* react in a very similar way to them. These two barleys are susceptible (reaction type 3+4− or 4) to all the races except races 4, 5 and 6, to which they are moderately resistant (reaction type 2 3 or 2). Moreover, they are of no value in differentiating races 4, 5 and 6 from each other.

On the contrary, Gassner & Straib's barley Aegyptische 4-zeilige Sommergerste, added by Ronsdorf to Hey's selection, proved to be the key to differentiation between some physiologic races, and it is quite indispensable in differentiating race 24 from 12; with these two races it gives reaction types 0 and 3+4−, 4, respectively.

Two of Mains's barleys also proved to be of great value. Quinn C.I. no. 1024 was the only barley on which physiologic races 16 and 17 could be differentiated. It gave a reaction type 0 with the latter and 2–3 with the former. Also, race 18 could be distinguished from race 22 on this barley, the former giving reaction 4 and the latter 1−, 2−. Lastly, Quinn C.I. no. 1024 was useful in distinguishing races 20 and 22. Bolivia C.I. no. 1257 was the only barley on which races 21 and 23 could be differentiated. Race 21 gave a reaction 2, 3− and race 23 gave a reaction 0, 2−. Unfortunately, this barley is of little value for differentiating the other races, since it often shows the heterogeneous reaction X. However, it must be kept in the differential set until a better variety is found.

For the purpose of differentiating the thirty physiologic races of *P. anomala* Rost. now known the following barleys are suggested:

Breustedts Schladener
Hordeum vulgare speciale
Friedrichswerther Berg Wintergerste
Australische Recka
Lichtis Lechtaler
Samaria 4-zeilige
Hordeum vulgare pallidum (Sudan)
Aegyptische 4-zeilige Sommergerste
Quinn C.I. no. 1024
Bolivia C.I. no. 1257

Oderbrucker C.I. no. 940 may also be kept as a subsidiary variety for differentiating the two groups of races, as suggested by Ronsdorf, which are differentiated by reactions 0–1 and 3–4.

Although this new arrangement of differential hosts makes possible a much better differentiation of the races much work remains to be done on this subject. First it seems necessary to carry out an investigation on a large collection of barleys, using not only the well-differentiated physiologic races, but also certain strains, now grouped under the same race which give slight, but constant, different reactions. Such slight differences were emphasized for cultures of races 12 and 13. These differences in reaction have been regarded as merely satellite variations, but in future they may prove to be due to different physiologic races not differentiable on the basis of our actual host range.

Bruens Neuzucht 25 was chosen by Stakman *et al.* (1935) as a differential host on the basis of an analysis of Hey's results (1932). It gave reaction type 4 with races 1, 2, 3, 4 and 5 and reaction 1 with race 6. This barley should be tested with all the available races to ascertain its real value as a differential host.

The six barleys, Gold, Flinn, California Feed, Stavropol, Chile and Odessa, used by Brown (1931) for differentiation of four Canadian physiologic races of brown rust of barley, should also be tested in order to determine whether any of the European races may be identified with the Canadian ones, and to establish the merits of these barleys as differential hosts.

The reaction types of the thirty physiologic races on the new set of barleys are given in Table V and an analytical key is provided in Table VI. In Table V only the extreme readings obtained during the experiments with physiologic races 12–30 are given. For the first eight races, the reactions were taken from Hey's (1932) results on Breustedts Schladener. *Hordeum vulgare speciale*, Friedrichswerther Berg Wintergerste, Australische Recka, Lichtis Lechtaler, Samaria 4-zeilige and *H. vulgare pallidum* (Sudan) at mean temperatures of 18 and 25° C. The reactions on Aegyptische 4-zeilige Sommergerste, Quinn C.I. no. 1024, Bolivia C.I. no. 1257 and Oderbrucker C.I. no. 940 of races 2, 3, 4, 5 and 9 were obtained from Ronsdorf's papers (1934, 1935): on Aegyptische 4-zeilige Sommergerste the reactions were taken by Ronsdorf at mean temperatures of 18 and 25° C. (1934), and on the other barleys the reactions were taken at mean temperatures of 12 and 22° C. (1935).

Reactions of races 9, 10 and 11 on Hey's standard barleys were also obtained from Ronsdorf's papers. The readings were taken at 18 and 25° C. for races 9 and at 26° C. for races 10 and 11.

Owing to differences in temperature at which the experiments have been carried out it is difficult to compare the results in some cases. However, this difficulty could not be overcome, as some of the races were described in different countries under diverse environmental conditions.

Distribution and prevalence of physiologic races of *P. anomala* in Great Britain, Spain and Portugal

The present account of the distribution of physiologic races of *P. anomala* Rost. must be regarded as a preliminary survey. Material was obtained from Great Britain, Spain and Portugal, but the localities in these countries were not sufficiently scattered for a comprehensive survey. Thus, with the English material thirty-eight collections were tested but thirty-four of these were collected at Cambridge.

Table V. *Pathogenicity of physiologic races of* P. anomala Rost. *on eleven differential barleys*

Physiologic races	Breustedts Schladener	*Hordeum vulgare speciale*	Friedrichswerther Berg Wintergerste	Australische Reeka	Lichtis Lechtaler	Samaria 4-zeilige	*Hordeum vulgare pallidum*	Aegyptische 4-zeilige	Quinn C.I. no. 1024	Bolivia C.I. no. 1257	Oderbruker C.I. no. 940
1	0, 1	0, 1	0, 1	1·2	4, 3	2·3, 2	3·4				
2	3·4	3·4	3	1·2	4, 3	2, 3	1·2	1, 2·3	0·1, 0	2, 3·2	4
3	3·4	3·4	3·4	1·2	4, 3	2·3, 2	3·4	3	1, 0	1·2, 2·3	4
4	3·4, 3	3·4, 3	3·4, 3	0·1	4, 3	1, 2	3·4	2, 3·4	0	2	4
5	3·4, 3	3·4, 3	3	1·2, 1	4, 3	1, 2	3·4,	1, 2·3	0·1, 0	2	4
6	3·4, 3	3·4, 3	3·4, 3	1·2, 1	1, 2·3	2·3, 2	1·2, 1				
7	0, 1	0, 1	0, 1	1·2	1·2, 2	3·2	3·4				
8	0, 1	0, 1	0, 1	3·4, 4	1·2, 2		3, 3·4				
9	3·4	4	3·4	3·2, 3·4	3·4	3·2, 3·4	3	3, 3·4	2·1, 0	1 2, 2 3	4
10	0·1	0	1	3·2	2·1	1·3	3·4				
11	3·2	3·2	4·3	3	4·3	3·4	4·3				
12	4 –, 4	4	3 + 4 , 4	3 + , 4	3 , 4	3 + 4 –, 4	4	3 4 , 4	3, 4	4 , 4, 4X	4 , 4
13	4	4	3 + 4 –, 4	1, 2	3, 4	4	4	3 4 , 4	3, 4	4, 4X	4
14	1, 4 –	1 +, 4X	2, 4 –, X	2, 4	3 4 , 4	4 –, 4	3 , 3	2, 3	2, 3 + 4 –	3 + 4 , 4X	1·3 +
15	0, 1	0, 1	0, 1·2	3·4	4	3 + 4 , 4	4	3, 4	1, 2 3	3, 4, X	0, 1·2
16	0, 2	0, 2	0, 2	0, 2	3 4 , 4	4	4	0, 2	2, 3	3X, X	0, 2
17	0, 2	0, 2	0, 2	0 +, 2	3 4 , 4	4	4	0, 1 2		3, X	0, 2
18	0, 1	0, 1	0, 1	4	4	4	4	4	4	3 , 4	0·1 +
19	0, 1	0, 1	4	4	2 3	4	4	4	4	4	1
20	1	0	1	4 –, 4	2	4	4	4	4	3	2
21	0	0	0	3·4	4	4	4 –	2 , 3	3 4	2, 3	0
22	1 +	0, 1	1 +	4	4	2 , 2	4	4 , 4	1 , 2	4	1, 2
23	0, 1	0, 1 –	0, 1	4 , 4	3 + 4 , 4	4	4	2 , 3, 3X	2 , 4	0, 2	0, 0·1
24	4	4	4	4	3, 3 +	4	4	0	2 3 , 3	4, X	4
25	0, 0·1	4 –, 4	0·1, 1	4	3 + 4	4 , 4	4	4	1 2, 2	4 , 4X	4
26	0	0	0, 1 –	4	2 , 2 + 3	4	4	4, 4X	2	4	1
27	4 –, 4	4	4	0, 0·1	2	4	4	4	2, 2	4	0, 0·1
28	0, 1 +	4	0, 1	4	3 + 4	4	4	4	4	4	4
29	4	4	4	2	3 + 4	4	2	4, X	1 , 2	4	4
30	0, 1	0, 1	0, 1 +	3 + 4 –, 4	3 + 4 , 4	4	4	0, 1	0, 1	4, 4X	1
							A	Mains's physiologic races	0, 2	0, 0·2	0, 1
							B	,, ,,	0, 0·1	0, 0·1	3·4, 4

Table VI. *Analytical key for the identification of physiologic races of* P. anomala *Rost. showing their pathogenicity on ten differential barleys*

Differential hosts and their behaviour	Physiologic races
Breustedts Schladener—resistant to moderately resistant (0·2)	
Hordeum vulgare speciale—resistant to moderately resistant (0·2)	
Friedrichswerther Berg—resistant to moderately resistant (0·2)	
Australische Recka—resistant to moderately resistant (0·2)	
Lichtis Lechtaler—resistant to moderately resistant (1·2)	7
Lichtis Lechtaler—susceptible (3 + 4 −, 4)	
Samaria 4-zeilige—moderately resistant to moderately susceptible (2·3)	1
Samaria 4-zeilige—susceptible (4)	
Quinn C.I. no. 1024—immune (0)	17
Quinn C.I. no. 1024—resistant to moderately susceptible (1·3 −)	16
Australische Recka—moderately resistant to moderately susceptible (3·2)	10
Australische Recka—susceptible (4)	
Lichtis Lechtaler—resistant to moderately resistant (1·2)	8
Lichtis Lechtaler—moderately resistant to moderately susceptible (2 −, 2 + 3 −)	
Quinn C.I. no. 1024—moderately resistant (2)	26
Quinn C.I. no. 1024—susceptible (4)	20
Lichtis Lechtaler—susceptible (3 + 4 −, 4)	
Bolivia C.I. no. 1257—resistant to moderately resistant (0·2 +); yellow pustules	23
Bolivia C.I. no. 1257—moderately resistant to moderately susceptible (2·3)	21
Bolivia C.I. no. 1257—susceptible (3 + 4 −, 4)	
Aegyptische 4-zeilige—resistant (0·1)	30
Aegyptische 4-zeilige—susceptible (3 + 4 −, 4)	
Quinn C.I. no. 1024—resistant to moderately susceptible (1 −, 2·3)	
Samaria 4-zeilige—moderately resistant (2 −, 2)	22
Samaria 4-zeilige—susceptible (4)	15
Quinn C.I. no. 1024—susceptible (4)	18
Friedrichswerther Berg—susceptible (4)	19
Hordeum vulgare speciale—susceptible (4)	
Quinn C.I. no. 1024—resistant to moderately resistant (1·2, 2)	25
Quinn C.I. no. 1024—susceptible (4)	28
Breustedts Schladener—moderately resistant to moderately susceptible (3·2)	11
Breustedts Schladener—susceptible (3·4, 4)	
Australische Recka—resistant to moderately resistant (0·2)	
Lichtis Lechtaler—resistant to moderately resistant (1, 2·3)	
Hordeum vulgare pallidum—resistant (1·2)	6
Hordeum vulgare pallidum—susceptible (4)	27
Lichtis Lechtaler—susceptible (3 + 4 −, 4)	
Quinn C.I. no. 1024—resistant (0·2 −)	
Bolivia C.I. no. 1257—resistant to moderately susceptible (1, 2·3)	
Hordeum vulgare pallidum—resistant to moderately resistant (1·2)	2
Hordeum vulgare pallidum—susceptible (3·4)	
Samaria 4-zeilige—resistant to moderately resistant (1, 2)	
Aegyptische 4-zeilige—moderately resistant to moderately susceptible (1, 2·3)	5
Aegyptische 4-zeilige—moderately resistant to susceptible (2, 3·4)	4
Samaria 4-zeilige—moderately resistant to moderately susceptible (2, 3)	3
Bolivia C.I. no. 1257—susceptible (4)	29
Quinn C.I. no. 1024—moderately susceptible to susceptible (3, 4)	13
Australische Recka—moderately susceptible to susceptible (3·2, 4)	
Aegyptische 4-zeilige—resistant (0)	24
Aegyptische 4-zeilige—moderately resistant to susceptible (3, 4)	
Quinn C.I. no. 1024—resistant (0, 2)	9
Quinn C.I. no. 1024—susceptible (3 + 4 −, 4)	12

The present results were obtained from seventy-seven collections of *P. anomala*: thirty-eight from five localities in Great Britain: Cambridge, Cardiff, Reading, Truro and Hartford (Northumberland); six from three regions in Spain: Madrid, Jerez de la Frontera and Zaragoza; and the remaining thirty-three from scattered places in Portugal (at or near: Lagos, Alvito, Barreiro, Lisbon, Cintra, Cascais, Leiria, Tomar, Coimbra, Porto, Santo Thirso, Pedras Salgadas, Chaves and Bragança). Eleven new physiologic races were identified and numbered 12–22. The distribution of these physiologic races in the three countries was as follows.

Great Britain:

Race 12 was found at Cambridge. Culture 1 was obtained from the Botany School, and cultures 35, 36, 39, 41, 42 and 44 were obtained from the University Farm.

Race 13 was found near Cardiff and at Cambridge. Culture 32 was obtained from Cardiff, cultures 10–31 were obtained from the Cambridge Botanic Gardens, and cultures 37, 38, 40 and 43 from the University Farm.

Race 14 (culture 34) was obtained from Hartford, Northumberland.

Race 15 was obtained from the Cambridge University Farm (culture 2) and from Truro, Cornwall (culture 33).

Race 18(?), culture 9, was obtained from Reading.

Spain:

Race 12 was found in material sent in 1936 from Jerez de la Frontera (cultures 69 and 70) and from Zaragoza (culture 71).

Races 16 and 17 were found mixed in a sample of material sent in 1933 from Madrid (Campo de Moratalá). Race 15 was identified in cultures 3 and 7 and race 17 in cultures 4, 5, 6 and 8.

Race 21 (culture 68) was obtained in 1936 from material sent from Madrid.

Race 22 (culture 82) was found in another sample sent from Madrid.

Portugal:

A wider survey has been possible in this country during the years 1935 and 1936.

Race 12 was found to be widely distributed over the country from north to south. It was isolated from Lagos (culture 48), Alvito (culture 57), Barreiro (culture 59), Lisbon (cultures 45, 46, 47, 50, 61, 62, 63, 64 and 65), Cascais (cultures 49 and 66), Cintra (culture 60), Leiria (culture

56), Tomar (cultures 51, 52, 53, 54 and 55) and Porto (culture 72). However, this race was not isolated from any sample sent from the north-eastern part of the country.

Race 18 was isolated from two samples, one from Santo Thirso (culture 58), the other from Pedras Salgadas (culture 67).

Races 19 and 20 were both found in a single collection from Coimbra (cultures 73 and 74).

Race 21 was confined to the north-eastern country of Traz-os-Montes. It was found in samples sent from Chaves (cultures 75–80) and from Bragança (culture 81).

Field observations on the occurrence of *P. anomala*

There is evidence that *P. anomala* may overwinter in East Anglia either in its uredospore stage or as dormant mycelium. This opinion is founded on field observations made in Cambridge during the years 1933 and 1934. During these winters scattered tiller shoots and seedlings bearing uredo pustules of *P. anomala* were continually found. The rust was abundant on these scattered plants until the end of November. During December it seemed that new infections by means of air-borne spores were more or less checked. In January, February and March only rare, small, single pustules could be detected, chiefly on the leaf sheaths. It seems probable that these scattered infections are sufficient to maintain the uredosporic cycle of this rust during the winter. It is the simplest explanation of the continuation of the rust, since I have never been able to find the aecidial stage of *P. anomala* in England although continuous search has been made for it. April seems to be the most favourable period for the dissemination of the rust spores and in May it is already common.

Under Portuguese conditions, in Lisbon and suburbs, there is no doubt that overwintering of the rust in the uredospore stage commonly occurs. But there the problem is, how does the rust survive the summer? In the hottest months no barleys or tiller shoots can withstand the long drought, and the question remained unanswered until the late summer of 1935. While at Cintra, numerous barley plants were found bearing uredo pustules of this and other rusts. Cintra is high above sea level and the plants were growing freely on the edges of the woodlands on the margins of allotment gardens. The rust was also found at sea level in Colares near brooks and creeks in shady places.

Had a detailed survey been made it is possible that a similar summer distribution of uredospores would have been observed in other places.

The observations at Cintra are significant because they show that the rust may pass the summer successfully in localized cool, sheltered places. As soon as the first rains begin in early autumn new barley plants grow from the fallen grains, providing for the propagation of the rust. From such altitudes the spores are carried by wind to the plains in central Portugal where most of the winter cereals are grown.

This explanation agrees with Mehta's (1933) view on the maintenance of cereal rusts in India. It also seems a more probable explanation than the infection of barley by aecidiospores. Although *Ornithogalum umbellatum* is fairly common in Portugal, no single natural infection of *Aecidium ornithogalum* has been found. In support of this view there is also the fact that near Lisbon the only prevalent physiologic race is no. 12, which from English material proved to be heterozygous. If the aecidial stage developed regularly every year in this region, to maintain the rust through the summer, it would most probably give rise to other physiologic races. However, no other physiologic race could be found either at Cintra, Cascais or Lisbon.

Summary

1. Methods of establishing pure cultures of rusts are discussed and a new spore-proof and insect-proof cellophane cylinder is described.

2. Eighty-two cultures, isolated from collections of *P. anomala* in Great Britain, Portugal and Spain, have been tested for the determination of physiologic races. The barleys used as differential hosts were those used by Hey and Mains and also Aegyptische 4-zeilige Sommergerste. Eleven new physiologic races have been identified and are numbered 12 22. Race 12 was found to be widely distributed in Great Britain, Portugal and Spain, the other ten were localized. Races 13, 14, 15 and probably 18 were found in Britain, races 18, 19 and 20 in Portugal, and races 16, 17, 21 and 22 in Spain.

3. A mutant, differing in colour (orange and yellow) and in pathogenicity, is recorded for *P. anomala*. The mutant arose from an unstable culture of race 14 cultivated on *Hordeum vulgare pallidum* (Sudan), and is numbered physiologic race 23.

4. Segregation and hybridization of physiologic races occurred through the aecidial stage on *Ornithogalum umbellatum* L. The heterozygous condition of physiologic races 12, 13 and 23 was demonstrated. Self-fertilized material from race 12 gave rise to the parent race, to races 16(?) and 19 and to four new races described under the numbers 24, 25, 26 and 27. From self-fertilized material of race 13, races 12(?), 13, 22 and 24

were isolated and also two new races numbered 28 and 29. From self-fertilized material of race 23, races 12, 19, 20 and 22 were isolated. A reciprocal cross between physiologic races 13 and 23 was achieved; in one direction the progeny gave rise to a single physiologic race, which proved to be new and is numbered 30; in the other direction the two physiologic races 30 and 18 were produced. This result is discussed and is attributed to cytoplasmic inheritance.

5. Several biotypes of physiologic races 12 and 13 were found; they seem to establish a linkage between the two races.

6. A new selection of differential hosts for the differentiation of physiologic races of *P. anomala* on cultivated barleys is proposed. A "Numerical Table" of the thirty physiologic races and an "Analytical Key" for their determination are given.

7. The distribution of physiologic races of *P. anomala* on cultivated barleys in Great Britain, Portugal and Spain is discussed.

8. From field observations it is concluded that at Cambridge *P. anomala* may overwinter in its uredospore stage. Evidence is given that in Portugal the uredospores survive the summer in the mountains.

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REFERENCES

Brown, A. M. (1931). Investigations on the dwarf leaf rust of barley (*Pucc. anomala*). Report of Dominion Botanist for 1930. *Rep. Dep. Agric. Can.* p. 56.

Cotter, R. U. & Levine, M. N. (1932). Physiologic specialization in *Puccinia graminis secalis*. *J. agric. Res.* **45**, 297.

Craigie, J. H. (1927*a*). Experiments on sex in rust fungi. *Nature, Lond.*, **120**, 116.

—— (1927*b*). Discovery of the function of the pycnia of the rust fungi. *Nature, Lond.*, **120**, 765.

—— (1928). On the occurrence of pycnia and aecia in certain rust fungi. *Phytopathology*, **18**, 1005.

—— (1931). An experimental investigation of sex in the rust fungi. *Phytopathology*, **21**, 1001.

GASSNER, G. & STRAIB, W. (1932). Über Mutationen in einer biologischen Rasse von *Puccinia glumarum tritici* (Schmidt) Erik. und Henn. *Z. indukt. Abstamm.-u. VererbLehre*, **63**, 154.

GOLDSCHMIDT, W. (1928). Vererbungsversuche mit den biologischen Arten des Antherenbrandes (*Ustilago violacea* Pers.). *Z. Bot.* **21**, 1.

HEY, A. (1932). Beiträge zur Spezialisierung des Gerstenzwergrostes, *Puccinia simplex* Erik. et Henn. *Arb. biol. Abt.* (*Anst.—Reichsanst.*), *Berl.*, **19**, 227.

HIRCHHORN, J. (1933). Dos royas de la cebada, neuvas para la Argentina. *Rev. Fac. Agron. La Plata*, **19**, 390.

JOHNSON, T., NEWTON, M. & BROWN, A. M. (1934). Further studies of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Pucc. graminis tritici*. *Sci. Agric.* **14**, 360.

MAINS, E. B. (1917). The relation of some rusts to physiology of their hosts. *Amer. J. Bot.* **4**, 179.

—— (1926). Studies in rust resistance. *J. Hered.* **17**, 313.

—— (1930). Host specialization of barley leaf rust, *Pucc. anomala*. *Phytopathology*, **20**, 873.

MAINS, E. B. & JACKSON, H. S. (1926). Physiologic specialization in the leaf rust of wheat, *Pucc. triticina* Erik. *Phytopathology*, **16**, 89.

MAINS, E. B. & MARTINI, M. L. (1932). Susceptibility of barley to leaf rust (*Pucc. anomala*) and to powdery mildew (*Erysiphe graminis hordei*). *Tech. Bull. U.S. Dep. Agric.* no. 295.

MEHTA, K. C. (1933). Rust of wheat and barley in India. *Indian J. agric. Sci.* **3**, 939.

MURPHY, H. C. (1935). Physiologic specialization in *Puccinia coronata avenae*. *Tech. Bull. U.S. Dep. Agric.* no. 433.

NEWTON, M. & JOHNSON, T. (1932). Studies in cereal diseases. VIII. Specialization and hybridization of wheat stem rust, *Puccinia graminis tritici*, in Canada. *Bull. Dep. Agric. Can.* no. 160, N.S.

NEWTON, M., JOHNSON, T. & BROWN, A. M. (1930*a*). A preliminary study on the hybridization of physiologic forms of *Puccinia graminis tritici*. *Sci. Agric.* **10**, 725.

—— —— —— (1930*b*). A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. *Sci. Agric.* **10**, 775.

PELTIER, G. (1925). A study of the environmental conditions influencing the development of stem rust in the absence of an alternate host. III. Further studies of the viability of the uredospores of *Puccinia graminis tritici*. *Res. Bull. Neb. agric. Exp. Sta.* no. 34.

ROBERTS, F. M. (1936). The determination of physiologic forms of *Puccinia triticina* Erik. in England and Wales. *Ann. appl. Biol.* **23**, 271.

RONSDORF, L. (1934). Einige Versuche über biologische Rassen des Gerstenzwergrostes. *Arb. biol. Abt.* (*Anst.—Reichsanst.*), *Berl.*, **21**, 109.

—— (1935). Weitere Untersuchungen über den Nachweis biologischer Rassen des Gerstenzwergrostes, *Puccinia simplex* Erik. und Henn. *Phytopath. Z.* **7**, 236.

STAKMAN, E. C. (1930). Racial specialization of parasitic fungi. *Trans. Ky Acad. Sci.* **3**, 93.

STAKMAN, E. C. & LEVINE, M. N. (1922). The determination of biologic forms of *Puccinia graminis* on *Triticum* sp. *Tech. Bull. Minn. agric. Exp. Sta.* no. 8.

—— —— —— (1935). Analytical key for the identification of physiologic forms of *Puccinia graminis tritici*. From division of cereal crops and diseases. *U.S. Dep. Agric. Minn. agric. Exp. Sta.*

STAKMAN, E. C., LEVINE, M. N. & COTTER, R. U. (1930). Hybridization and mutation in *Puccinia graminis*. *Phytopathology*, **20**, 113.

Stakman, E. C., Levine, M. N., Christensen, J. J. & Isenbeck, J. J. (1935). Die Bestimmung physiologischer Rassen pflanzenpathogener Pilze. *Nova Acta Leop. Carol.* **13**, 319.

Vavilov, N. I. (1919). Immunity of plants to infectious diseases. *Bull. Petrovsk. Acad.* (1918). (English résumé.)

Ward, H. M. 1902 . [illegible] *Proc. Roy. Soc.* **69**, 451.

—— (1903). Further observations on the brown rust of the Bromes (*Puccinia dispersa* Erik.) and its adaptive parasitism. *Ann. mycol., Berl.*, **1**, 132.

Waterhouse, W. L. (1927). Studies in the inheritance of resistance to leaf rust, *Puccinia anomala* Rost., in crosses of barley. *J. roy. Soc. N.S.W.* **61**, 218.

—— (1929). Australian rust studies. *Proc. Linn. Soc. N.S.W.* **54**, 615.

Postscript. Further papers by W. Straib on *Puccinia anomala* (*Arb. biol. Abt. (Anst.—Reichsanst.), Berl.* (1937), **22**, 43 and *Züchter* (1937), **9**, 304) were received too late for consideration in connexion with my results.

B. d'Oliveira.

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CONIOPHORA PUTEANA (SCHUM.) KARST. ON LIVING *SEQUOIA GIGANTEA*

By J. A. MACDONALD

Botany Department, St Andrews University

(With Plate IV)

Trees of *Sequoia gigantea* growing in a garden in St Andrews were cut down in the spring of 1936. A piece of timber measuring approximately 30 in. in diameter and 12 in. in thickness from the base of a trunk was brought to the Botany Department. The annual rings exposed in cross-section showed the age of the tree to be 36 years. It was noted that there were considerable pockets of rotted wood in the outer portions at one side of the trunk, these being clearly visible on both transverse faces. Using some of this infected material as inoculum cultures were made in the manner described below. The rotted areas, which showed cubical cracking of the attacked wood, varied in size up to a maximum of approximately 5 in. by 2 in. The portion of trunk was left beside a potting shed till the autumn, in order to allow the wood to dry out. It was then planed smooth on the lower side and photographed to show the rotted portions (Pl. IV, fig. 1).

The fungus in culture

(*a*) *On agar*. The only organism isolated from the rotted areas was brought into culture on 2½ % malt agar. Thin pieces of wood not exceeding 5 mm. in length were taken at random from the rotted areas. They were either washed with 1% hydrochloric acid or dipped in methylated spirit and flamed before being placed on the surface of malt agar slopes contained in 6 in. test-tubes. After 7 days several of the cultures showed hyphae growing out from the inoculum on to the surface of the agar. The mycelia formed subsequently were all similar in appearance and continued to exhibit the same range of characters in all transfers.

Growth rate and type were studied on malt, prune and potato-dextrose agars. On the last-named medium the characters exhibited by the fungus were such that it fitted into Fritz's (1923) key for the identification of wood-destroying fungi along with the fungus causing Balsam rot type A. The principal characters used in making this determination

were as follows. Growth at first white, silky, with prominent strands; becoming denser, woolly, with tangled, loose hyphae above the mat. Colour white, rapidly becoming tinged yellow; finally yellow more or less patched with brown, particularly where the medium tends to dry out. Slope covered in 10–14 days at 22° C. The hyphae bear single, paired and whorled clamp connexions. "Finger hyphae" were observed frequently, as figured by Kemper (1936). Fritz (1923) concluded that her cultures might be *Coniophora cerebella* (*puteana*), but pointed out that they differed from earlier descriptions of that fungus in the absence of oidia (Möller, 1907). In the cultures obtained from *Sequoia* oidia were freely produced, and when old cultures were opened they were on occasion set free as visible white clouds. They appeared to be present in all cultures more than 14 days old. The growth rate of colonies in tube culture, on malt agar, was calculated over a period of 12 days. The figures obtained represented an average increase of 2·3 cm. in 2 days. This agrees well with the figure of 2·4 cm. given by Falck (1909) for *Coniophora cerebella* (*puteana*). No oxidation rings were formed when the fungus was grown on a nutrient medium with additions of 0·5 % or 0·25 % tannic acid. This agrees with Bavendamm's (1936) findings for *C. cerebella* (*puteana*) and other fungi of his cellulose-attacking type. Attempts to induce the production of fructifications either on agar or wood blocks were unsuccessful. Nevertheless, the close agreement between the cultures derived from the infected *Sequoia* and cultures and descriptions of *C. cerebella* (*puteana*) leaves no doubt that they are the same.

(*b*) *On wood blocks*. Small blocks of various types of wood were placed on cotton-wool plugs in boiling tubes. The tubes were filled one-third full with water and then sterilized. Afterwards the wood blocks were inoculated on the upper end with mycelium from malt-agar culture. Blocks of sound *Sequoia* wood from both inner and outer regions of the trunk were employed. In addition the following woods were infected: lime, maple, horse-chestnut, hornbeam, alder, oak, elm, beech, willow and birch. The sequence corresponds with the declining order of the luxuriance of the mycelium produced on the respective woods. In all cases the growth was more vigorous than that produced on *Sequoia*. Characteristic mycelial strands were a prominent feature in the earlier stages of growth (Pl. IV, fig. 2).

Brown zone lines were formed on the surface of some of the wood blocks and against the glass of the containing tubes. In addition, irregularly shaped, dark brown structures appeared on the thicker mycelial threads. The largest of these reached a diameter of 2 mm. Many were not

visible individually with the naked eye, but were easily distinguished with a hand lens. They were composed of aggregates of thick-walled, brown pieces of hyphae and may be regarded as sclerotioid in nature.

The type of rot produced in culture is characterized by the appearance of horizontal cracks in the wood (Pl. IV, fig. 3). This is similar to the naturally produced rot. The loss in weight of artificially infected *Sequoia* blocks was estimated at approximately 43% of the fresh weight after 9 months, and that of the control blocks at approximately 26%, making the loss due to the activity of the fungus 17% approximately.

The mycelium in both naturally and artificially infected wood was similar in appearance. It was readily picked out in sections stained by Cartwright's (1929) method. Hyphae were numerous, running along the wood elements and penetrating the lateral walls by means of the bordered pits and by bore holes. Clamp connexions and intercalary chlamydospores were observed.

PARASITISM OF THE FUNGUS

Fritz (1923) found a *Coniophora* associated with *Polyporus balsameus* in cubical rots of *Abies Balsamea* and *Picea mariana* in Canada. She was of the opinion that the former fungus was present as a secondary parasite. Meyer (1934) has recorded *Coniophora cerebella* on living birch near Moscow, maintaining itself in enclosed knots in the wood and subsequently rotting the felled timber. Robak (1936), examining brown cubical butt rots of spruce in Norway, isolated a *Coniophora* only and thought that it was of primary importance. In Britain, Day & Peace (1936), studying butt rot of conifers, state that *C. cerebella* (*puteana*), among other fungi, may be associated with this condition. European Larch and Norway Spruce are commonly affected and Scots Pine rarely; while Douglas Fir, Sitka Spruce, Japanese Larch, *Thuja* sp. and Lawson's Cypress have all been found seriously attacked.

As far as can be ascertained this is the first record of a *Coniophora* on *Sequoia gigantea*. In the present instance the fungus was the only organism isolated from the rotted areas of the trunk immediately after felling. In these circumstances it seems clear that it was acting as a primary parasite.

The increasing number of records of *Coniophora puteana* on living wood serves to draw attention to an important source of infection of timbers which may be used in constructional work.

SUMMARY

1. When a 36-year-old tree of *Sequoia gigantea* was felled it was found to have rotted areas in the wood at the base of the trunk.

2. One organism only was isolated from wood in these rotted areas.

3. The general and detailed microscopic characters exhibited by this fungus in culture identify it as *Coniophora puteana*.

4. In the present case it seems clear that the fungus was acting as a primary parasite.

REFERENCES

BAVENDAMM, W. (1936). Erkennen, Nachweis und Kultur der holzverfärbenden und holzzersetzenden Pilze. *Hand. biol. ArbMeth.* Abt. **12**, 927.

CARTWRIGHT, K. ST G. (1929). A satisfactory method of staining fungal mycelium in wood sections. *Ann. Bot., Lond.*, **43**, 412.

DAY, W. R. & PEACE, T. R. (1936). Butt rot of conifers. *Scot. For. J.* **50**, 52.

FALCK, R. (1909). Die Lenzitesfäule des Coniferenholzes. *Hausschwammforsch.* **3**, 1.

FRITZ, C. W. (1923). Cultural criteria for the distinction of wood-destroying fungi. *Trans. roy. Soc. Can.* **17**, sect. v, 191.

KEMPER, W. (1936). Zur Morphologie und Zytologie der Gattung *Coniophora* (*usw.*) insbesondere des sogenannten Kellerschwamms. *Zbl. Bakt.* Abt. II, **97**, 100.

MEYER, E. I. (1934). Black knots and darkening of the heartwood in Birch in relation to the initiation of timber rotting. *Rev. appl. Mycol.* **14**, 269.

MÖLLER, A. (1907). Hausschwammuntersuchungen. *Hausschwammforsch.* **1**, 29.

ROBAK, H. (1936). Notes on Norwegian wood rots. II. The genus *Coniophora* DC. and the "*Vaporarius* rot" in conifers. *Nyt. Mag. Naturw.* **76**, 2.

EXPLANATION OF PLATE IV

Fig. 1. Cross-section of *Sequoia* trunk showing dark brown rotted areas towards the foot of the photograph.

Fig. 2. Birch block artificially infected with *Coniophora puteana*. Characteristic strands have formed on the surface of the bark.

Fig. 3. *Sequoia* wood block, artificially infected with *Coniophora puteana*, nine months after infection. Horizontal cracking of the wood can be seen.

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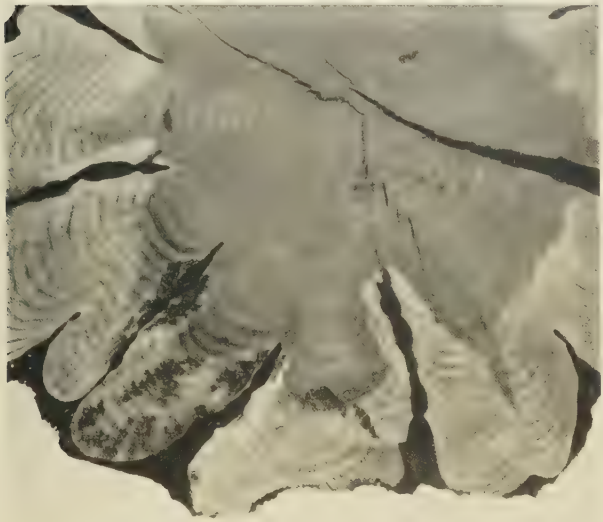

Fig. 1.

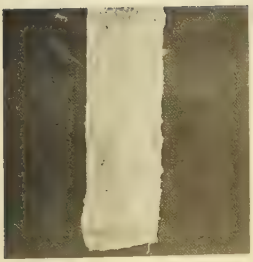

Fig. 2.

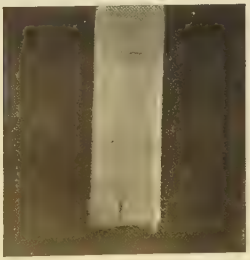

Fig. 3.

MACDONALD.—*Coniophora puteana* (Schum.) Karst. on Living *Sequoia gigantea* (pp. 83–86)

NOTES ON THE PHOTOPERIODIC REACTIONS AND VIRUS CONTENTS OF SOME PERUVIAN POTATOES

By R. W. G. DENNIS, B.Sc., Ph.D.[1]
The Potato Virus Research Station, School of Agriculture, Cambridge

(With Plates V and VI)

CONTENTS

I. Introduction

In December 1937 a consignment of fifty-nine potato varieties cultivated at Puño and collected there by the Percy Sladen Expedition to Lake Titicaca was received at the Potato Virus Research Station, Cambridge. Only the vernacular names of the varieties were supplied by the collectors and their botanical relationships have not been fully worked out. Two forms of Papa surimana, with pink and purple tubers respectively, appear to belong to *Solanum chaucha*; Azul parcco, Parcco caramo, Parcco hancco, Luqui mari and perhaps Pocco tturo huilla appear to form a homogeneous group so far unidentified, and most of the other varieties may provisionally be regarded as forms of *S. andigenum*.

The tubers were grown in an insect-proof greenhouse with a view to their multiplication and distribution as an adjunct to the Empire Potato Collection now being made by an expedition to Mexico and South America. During 1938 investigations were carried out to determine their photoperiodic reaction and their approximate virus content.

II. Influence of length of day on growth and yield

The influence of length of day on the growth of the domestic potato has been studied by Garner & Allard (1923) and Tincker (1925). The former found that in the case of the McCormick variety an increase in the period of illumination from the normal summer day (14-15 hr.) to 18 hr.

[1] Now at the Seed Testing and Plant Registration Station, East Craigs, Corstorphine, Edinburgh, 12.

completely inhibited tuber formation, while, although flower buds were formed, they did not open. When the length of day was artificially restricted the plants bloomed earlier than the controls, and with periods of 10 and 13 hr. illumination the ratio of tubers to top was greater than with the controls. With the 10 hr. day this ratio was at a maximum, but the actual yield per plant was less than in full daylight. These workers similarly found that tropical yams (*Dioscorea alata*) gave a higher yield of tubers with 10 or 12 hr. illumination than with full summer day, and that Peruvian and Bolivian varieties of bean (*Phaseolus vulgaris*) only flowered under conditions of 12 hr. days or less (Garner & Allard, 1920). Tincker, working with King Edward potato, found the optimum length of day for tuber formation to be 12 hr. Yields of plants receiving only 9 hr. light were greatly inferior to those from full summer day controls. The collection of potato varieties used in the present work was obtained from a locality in latitude 15° 53′ S., i.e. one where the length of day throughout the year varies approximately between 11 and 13 hr. Russian workers have shown that South American potato varieties collected from within the tropics do not yield well under the long-day conditions obtaining during summer at Leningrad, and that with them the optimum length of day for tuber formation is 9 hr.

The first crop of Peruvian potatoes raised at Cambridge was planted in December 1937 and harvested before the end of April 1938: thus growth was nearly completed before long-day conditions set in. A second group of tubers was potted in duplicate at the beginning of March. One set was given a 9 hr. day while the other was exposed to the full length of day experienced during summer at Cambridge (52° 12′ N.), varying between about 13 hr. at the beginning of April and over 16 hr. in mid-June. The experiment was intended merely to test the current view that Peruvian potatoes would not form tubers under long-day conditions, and estimation of yield was not contemplated. As only a small dark chamber was available the short-day plants were packed close together in 7 in. pots. The long-day plants were better spaced and had 8 in. pots and, contrary to expectation, most of them produced tubers. Direct comparison between yield obtained in the two sets is unprofitable, but in those cases where in spite of their more favourable surroundings the long-day plants yielded noticeably fewer tubers than the corresponding short-day ones, one seems justified in accepting the influence of length of day to explain the discrepancy.

The effect of long-day conditions on these "short-day" plants may be summarized as follows:

(1) *Effect on flowering.* Of the long-day series thirty five out of fifty-six flowered; of the short-day group only two varieties flowered, viz. Lanta mari and Hanco cuillo, and these did so about a fortnight later than the corresponding plants in the other set.

(2) *Effect on time of maturity.* As shown in Table I, the short-day plants matured over a period of about 1 month, after growing periods of 15–19 weeks. The long-day series, on the other hand, matured fairly uniformly during the second and third weeks of July.

(3) *Effect on tuber formation.* The effect on tuber formation involves two considerations, yield and dormancy. In Table I the varieties are classified under three main heads:

A. Those which produced good crops of normal tubers under long-day conditions.

B. Those which under the same conditions produced good crops in which the dormancy period was eliminated. This forms by far the largest group and may be subdivided into: (1) Varieties in which second growth occurred but in which the tubers did not send sprouts above ground. (2) Varieties in which the tubers produced long sprouts which came above ground to form erect green shoots, clustered round the base of the parent plant before the latter matured (Pl. V, fig. 4). The most extreme case in this group was Chachaza which formed eight primary tubers, each of which gave rise to a young plant bearing secondary tubers and stout aerial shoots. In other varieties tubers were produced by lateral buds, while the apex of the stolon grew for a long distance and finally turned up to form a leafy shoot. (3) Varieties in which the crop, though of fair quality, was less than that produced by the corresponding 9 hr. day plant.

C. Varieties in which tuber production was negligible under long-day conditions. Most of the plants produced a mass of long stolons which formed a felted mass round the edge of the pot and whose tips finally turned above ground as green shoots (group C (1)). Garner & Allard record production of somewhat similar offsets in McCormick potato subjected to 18 hr. illumination. In a small group (C (2)) consisting mainly of the "Parcco" varieties, stolon formation was almost inhibited. Oca (*Oxalis tuberosa*) and Papa lisa (*Ullucus tuberosus*) also fall in group C as regards tuber production.

It will be obvious that the groups outlined above are not to be regarded as hard-and-fast divisions. Thus, B (3) forms a transition zone between B and C, and it is possible that further experience may remove some of the varieties from group A to group B.

Table I. *Influence of length of day on tuber formation of Peruvian potatoes potted 2 March 1938*

Variety	Date of harvest	9 hr. day: No. of tubers	9 hr. day: Greatest length of tubers in.	9 hr. day: Wt. of tubers g.	Full day: No. of tubers	Full day: Greatest length of tubers in.	Full day: Wt. of tubers g.
Group A. Varieties yielding good crops of normal tubers under long-day conditions							
Alpaca noza	13. vii	10	1⅜	61	14	1⅞	208
Lanta mari	2. vii	10	1	32	32	1⅝	170
Cochicallo	15. vi	45	1⅝	91	20	2	94
						Average	157
Group B. Varieties yielding good crops of abnormal tubers under long-day conditions							
(1) Much second growth but no sprouting observed							
Papa ppitiquiña	15. vi	11	⅝	12	34	1¼	143
Condor huallcco	13. vii	16	1½	114	13	2½	158
Quero chiquilla	15. vi	23	⅞	49	28	2¼	120
Pichuya choque	15. vi	20	1⅜	80	31	2	187
Hanco cuillo	13. vii	22	1⅓	90	23	1½	152
Ppicochara	15. vi	22	1¾	63	16	2	87
						Average	141
(2) Good crops of tubers which produced aerial shoots before harvest							
Papa (black) surimana	13. vii	14	1	14	17	1⅞	87
Papa (red) surimana	2. vii	24	1⅜	60	39	1¾	193
Azul surimana	15. vi	22	1½	66	21	1⅞	126
Papa alcayurima	13. vii	31	¾	68	19	1½	140
Papa negra yanahimilla		11	1½	56	16	2	197
Panti imilla		11	1½	60	17	1¾	191
Pinta milagro	2. vii	23	1	60	25	1¾	216
Chuhuavisca	13. vii	12	1¾	62	46	1½	190
Puca ppitiquiña	13. vii	26	1¼	110	25	1½	208
Amajaya arama	15. vi	20	1¼	93	32	1¼	220
Yana imilla	2. vii	17	1	80	13	1½	105
Chupica imilla	2. vii	35	1	83	13	1¼	83
No. 162 Granja Salcedo	16. vi	22	1½	62	14	2	144
Chachaza	15. vi	14	1¼	60	8 + 7	1½	93
Sacampai choque	15. vi	27	2¼	64	19	1¾	98
Huilla mari	13. vii	8	1⅜	88	13	1⅜	136
Chohuahineca	13. vii	19	1⅜	77	37	2	157
Ppujno	13. vii	12	1¾	106	14	1¾	139
Cacho amajaya	15. vi	32	1¼	84	22	2¼	150
Huaca nuño	13. vii	33	1⅜	100	16	3	123
Chupica yarama	13. vii	15	1½	92	15	2½	155
Huilla imilla	15. vi	23	1	81	38	1⅜	162
Alcca fina	13. vii	26	2¾	116	25	2	221
Huaca huajra	13. vii	26	1¾	103	17	2⅛	136
Ccompis	16. vi	14	1¼	101	21	1¼	138
Chiquiña	15. vi	13	1⅜	61	9	1¾	69
Ruqqui	13. vii	14	2	100	16	1¾	155
Huaña	16. vi	16	1⅓	88	13	2⅛	224
Sihuanco choque	15. vi	22	1¾	65	23	1¾	113
Quello huaccoto	13. vii	45	1¼	127	11	1⅜	160
Futaco	13. vii	21	1¼	109	11	1¾	139
Ofrenta	16. vi	14	1½	96	25	2	233
						Average	154

Table I (*cont.*)

Variety	Date of harvest	9 hr. day: No. of tubers	9 hr. day: Greatest length of tubers in.	9 hr. day: Wt. of tubers g.	Full day: No. of tubers	Full day: Greatest length of tubers in.	Full day: Wt. of tubers g.
B (3) Poor crops of tubers which have produced aerial shoots							
Papa runtusa	16. vi	33	1	87	14	1¼	90
Phospa sunchus	16. vi	15	1½	91	6	1¾	44
Liquelique ppiqhe	15. vi	32	1	86	16	1¼	68
Ccosi	15. vi	20	2	103	16	1¾	95
						Average	74
Group C. Yield negligible or nil under long-day conditions							
(1) Stolons produced							
Papa ruqui	12. vii	16	½	15	3	¾	3
Papa negra	12. vii	35	¾	21	9	½	5
Chinamajaya	15. vi	29	1¼	64	1	1	8
Luqui mari	2. vii	10	1½	64	3	1	10
Milagro	15. vi	32	1¼	63	1	1½	8
Azul lajra	13. vii	19	1½	82	0	0	0
Poccoye	2. vii	34	1½	61	0	0	0
						Average	5
(2) No stolons produced (one short stolon in Azul parcco)							
Pocco tturo huilla	13. vii	5	1¼	28	0	0	0
Parcco hancco	13. vii	14	2¼	86	0	0	0
Parcco caramo	13. vii	13	1½	60	0	0	0
Azul parcco	13. vii	25	2	90	1	¾	5
						Average	1¼

From these data it seems that the varieties of groups A, B (1) and possibly B (2) could be grown in England with a prospect of fair tuber production, especially if they were planted early. Possibly had the tubers been planted 1 or 2 months later the adverse effect of long-day conditions might have been more marked. It was partly on a basis of their short-day habit that Russian authors considered the Peruvian potatoes unlikely ancestors of the domestic *S. tuberosum*, but it appears that this objection does not apply to all the varieties grown at Puño.

III. Virus content

The Peruvian potato varieties were subjected to the routine examination pursued with all new varieties received at the virus station. This involves sap inoculation to *Nicotiana tabacum* (variety White Burley), *Datura stramonium* and *Capsicum annuum* (variety Golden Dawn), and the grafting of unhealthy looking stocks to standard potato varieties such as Epicure, President, Arran Victory and Up-to-Date. The sap inoculations are sufficient to detect viruses *Y*, *X*, *F* and *G*, while suitable grafts reveal the presence of viruses *B*, *C*, *A* and leaf roll. In order to check the

presence of very weak strains of *X*, which may be carried by tobacco or *Datura* without evoking a mottle, it is customary to reinoculate such hosts with a severe "ring-spot" type of *X*. If infection does not follow reinoculation the presence of a weak or masked strain of *X* may be inferred (Salaman, 1933). It was not practicable to carry out a complete investigation of all the fifty-nine varieties, but all were tested by sap inoculation to tobacco, *Datura* and *Capsicum*, and the majority were also grafted to healthy President potato. Varieties whose virus content appeared of special interest were chosen for further investigation. The results may be summarized as follows:

(1) *Healthy varieties.* No sap-transmissible virus was found in the varieties Ccompis, Ccosi, Chinamajaya, Milagro, Papa negra, Phospa sunchus, Piñazo, Poccoye, Ppiscchara, Quero chiquilla and Sacampai choque, all of which appeared healthy under glasshouse conditions.

(2) *Virus X.* The presence of a virus resembling *X* was detected in twenty-eight varieties. In fifteen of these it appeared to be the only virus present, in nine it occurred in association with *B* and in two with a streak virus similar to *C*. In Alcca fina *X* was associated with a virus of the Aucuba group. In Chiquiña *X* was associated with a virus which killed Di Vernon by top necrosis. Of the varieties containing *X* alone, Chupica imilla, Granja Salcedo no. 162, Hanco ccillo, Huaña, Huilla mari, Liquelique ppiqhe, Papa ppitiquiña, Papa surimana (purple-tubered form) and Pichuya choque appeared healthy under greenhouse conditions, whilst Alpaca noza, Azul surimana, Huilla imilla, Ofrenta, Puca ppitiquiña and Ruqqui exhibited a mild interveinal mottle, most pronounced in the last named. The strains of *X* recovered were mostly of a mild type, giving rise to interveinal mottles on tobacco and *Datura* with no local lesions. Chiquiña, Granja Salcedo no. 162, Papa surimana and Ruqqui contained strains so weak as to evoke no symptoms on the above-mentioned plants, though capable of protecting them against reinfection with a stronger strain and of killing Epicure and Arran Crest by top necrosis. The Alcca fina and Huilla imilla strains induced faint ring-like local lesions in *Datura* (Pl. VI, fig. 5), whilst the form obtained from Pichuya choque and Azul surimana produced a few scattered yellow spots superimposed on the general mottle in tobacco. Attempts to isolate a ring-spot strain by punching out these yellow spots and inoculating them to fresh plants failed.

Papa ppitiquiña yielded an *X* which resembled Bawden's strain D (Bawden, 1934). On tobacco and *Datura* it gave rise to mild interveinal mottles only; on *Capsicum* grey local lesions appeared, followed by

systemic interveinal necrosis and die back. President and Arran Victory infected by graft or by sap developed a blotchy interveinal mottle with large rounded grey brown interveinal necroses and some leaf drop. King Edward was killed by top necrosis. A somewhat similar strain, inducing less severe foliar necroses on President, and interveinal mottle only on Arran Victory, was recovered from Puca puttiquiña. The virus of Alpaca noza gave rise to a rather bright interveinal mottle on President (Pl. V, fig. 1).

(3) *The Aucuba group.* Viruses inducing host reactions which recall those of the Aucuba or yellow mosaics of the potato (viruses *F* and *G*) were found in Alcca tina, Chachaza, Huaca huajra, Luqui mari, Panti imilla, Papa ruqui, Pareco caramo, Pocco tturo huilla, Ppuimo, Puca muru chimaco and Sihuanco choque. The general characters on which attribution to this group is based are: absence of symptoms on tobacco and *Datura* associated with failure to protect these plants against reinoculation with severe *X*, local lesions and systemic interveinal necrosis, sometimes followed by leaf drop and even die back on *Capsicum*, and failure to induce top necrosis in *X*-intolerant potato varieties such as Epicure and King Edward. In the case of Alcca tina, where a strain of *X* was also present, the occurrence of an Aucuba-type virus was inferred mainly from the reaction of Dunbar Yeoman. Symptoms in the original potatoes varied from none in the case of Huaca huajra, Panti imilla and Pareco caramo to more or less bright interveinal mottle in Alcca tina, Chachaza, Luqui mari, Papa ruqui, Ppaimo, Pocco tturo huilla and Puca muru chimaco. In the case of the third and sixth named the mottle was followed by roundish black interveinal blotches with irregular zoning of the target blotch type, leading ultimately to a dropping of the older leaves (Pl. VI, fig. 6). No fungus could be found in the foliar lesions nor could the symptoms be transmitted by grafting to standard British domestic potato varieties. Similar necrotic spots appear on the leaves of certain seedlings raised from the domestic potato and appear to bear no relation to their virus content. In Sihuanco choque a bright Aucuba-like mottle was observed.

Some difficulty is met with in the attempt to group these viruses under the two individuals *F* and *G* recognized by Clinch *et al.* (1936). Those from Panti imilla and Huaca huajra were carried without symptoms by Epicure, but the former gave rise to grey marginal necrosis on the lower leaves of Dunbar Yeoman and is therefore perhaps not a true Aucuba (*G*). The virus of Sihuanco choque, which induces an Aucuba mottle on its original host, and of Papa ruqui which was carried by Dunbar Yeoman

and induced mottlings and ruffling in King Edward, may be true *G*. The viruses of Alcca fina, Chachaza, Luqui mari, Pocco tturo huilla, Ppujno and Puca muru chimaco all induced marginal and terminal necrosis or scorching in Dunbar Yeoman leaflets and hence resembled *F* or tuber-blotch virus. A virus apparently identical with tuber blotch occurred associated with a streak in Parcco hancco (see §5(*c*)). Tuber symptoms were observed only with Puca muru chimaco virus on President, where faint internal brown spots were seen in one tuber out of four. It is possible that the examination of the tubers, made at the time of harvest, was premature, and that further symptoms of tuber blotch would have developed in them during storage.

(4) *Virus B*. The following potato varieties contained, in addition to *X*, a virus graft-transmissible to President, in which it caused top necrosis: Azul lajra, Cacho amajaya (Plant 1), Condor huallcco, Futaco, Huaca nuño, Papa alcayurima, Papa negra yanahimilla, Papa surimana (pink-tubered form) and Yana imilla. A similar virus, apparently free from *X* occurred in Azul parcco and Quello huaccoto. All these varieties appeared healthy with the exception of Azul lajra, Cacho amajaya and Futaco which showed a mild interveinal mottle. In the case of Papa alcayurima and Papa negra yanahimilla the virus also induced top necrosis in Arran Victory and was therefore probably Up-to-Date streak or virus *B*. The same conclusion may be reached for the Condor huallcco virus, which was carried by the U.S.D.A. seedling 41956, a variety usually killed by the other known streak, virus *C*. A second plant of Cacho amajaya, derived from a different tuber in the original consignment, appeared free from all viruses, as inferred from the results of grafts to Epicure and President.

(5) *Virus C*. Di Vernon streak or *C* has not been very fully studied, but the viruses present in the following varieties appear to resemble it, having distinctive characters in common with it:

(*a*) Amajaya arama and Pinta milagro. Both these varieties contained a mild *X* together with a virus which caused top necrosis on President but had no visible effect on Arran Victory.

(*b*) Huaca lajra was apparently free from *X*, *Y*, *F* or *G* since no symptoms resulted from inoculation to tobacco, *Datura* or *Capsicum*, but when grafted to President, Arran Victory, British Queen, Epicure and Majestic the stocks died of top necrosis. These reactions may be tentatively ascribed to the presence of both streak viruses, *B* and *C*, since although *B* can infect tobacco, *Datura* and *Capsicum*, it is not always readily sap-transmissible from potato to these hosts.

(*c*) Parcco hancco, which showed a rather fleeting veinal mottle of some upper leaves but had no other symptom of disease, contained a virus of the Aucuba group plus a streak virus similar to *C*. Sap inoculations to tobacco and *Datura* evoked no symptoms in the hosts nor were the tobaccos protected against reinoculation with severe *X*. Sap inoculation to *Capsicum* induced local lesions, systemic interveinal necrosis and leaf drop, indicating the presence of virus *F* or *G*. When grafted to healthy plants of U.S.D.A. seedling 41956 (two plants), President, Up-to-Date, Katahdin and Sharpe's Express, and to a President previously infected with a mild *X*, top necrosis resulted in the stock (Pl. V, fig. 2). Attempts to transmit the top necrosis virus by sap inoculation from the dying U.S.D.A. 41956 to healthy U.S.D.A. 41956, President, Arran Victory and Katahdin gave negative results. When two plants of Arran Victory were grafted from Parcco hancco their young leaves became slightly ruffled with interveinal mottle, while the intermediate and lower leaves exhibited numerous small interveinal necrotic spots and rings (Pl. V, fig. 3). The plants were not killed and the tubers showed no sign of necrosis. On Dunbar Yeoman there was no top necrosis but large interveinal necroses developed on the intermediate leaves, followed by general chlorosis and leaf drop. Streaks appeared on the stem and petioles but the growing point remained healthy.

On Epicure an interveinal mottle with light superficial necrosis of the upper leaves developed about a fortnight after grafting. Three weeks later all except the uppermost leaves were chlorotic and beginning to drop. After a further 10 days the shoots were all nearly dead, all the leaves were shrivelled and hanging, though still firmly attached to the stem. The tips had wilted but were not blackened as in typical top necrosis. These symptoms resemble strikingly those of tuber blotch on this variety as described by Clinch *et al*. (1936). In the case of Dunbar Cavalier the shoot next the Parcco hancco scion died of top necrosis, whilst two other shoots showed leaf drop, crinkling and light superficial foliar necroses only.

(6) *Possible new viruses*. In six Peruvian potato varieties, viruses or virus complexes were discovered whose reactions could not be readily interpreted in terms of the known potato viruses. The varieties were:

(*a*) *Papa runtusa*. This plant appeared healthy apart from a faint interveinal mottle on a few intermediate leaves. Sap inoculations from it to tobacco, tomato and *Lycium barbarum* led to no visible reactions, but the first-named plants were found to be protected against reinoculation with severe *X*. Sap inoculation to *Datura* did not result in local lesion formation, and for 16 days the inoculated plants remained healthy.

Some days later, however, the young leaves became deformed, with marked rugosity, faint brownish necrosis of the veins and a downward curling of the lamina (Pl. V, fig. 5). Five weeks after inoculation the leaves were waved, with a faint bright yellow veinal mottle and a few brownish necrotic specks on the finer veins. On *Capsicum annuum* brown local lesions were followed by systemic interveinal necroses.

Grafts from the original potato to Epicure (three plants) resulted in no well-defined disease, although the leaves of the stock were unusually dark green and glossy with slight waving. The scions grew well and formed good union with the stocks, while the presence of virus in the latter was demonstrated by grafting from them to healthy plants of President potato, which rapidly succumbed to top necrosis. Dunbar Yeoman similarly grafted developed large marginal necroses on the lower leaves, followed by general chlorosis and leaf drop. Grafted plants of Arran Victory, U.S.D.A. 41956 and Eclipse appeared unaffected by the viruses present. Reviewing the above data we might infer the presence of *F* or a related virus, from the Dunbar Yeoman and *Capsicum* reactions. The virus causing top necrosis of President cannot, however, be identified with either of the streak viruses known in this country, since *C* would have killed Eclipse and probably U.S.D.A. 41956, and *B* kills Arran Victory and Epicure.

(*b*) What seems a similar virus occurred in Chuhuavisca. Here again sap inoculation to tobacco produced no visible result at first, but the plants were protected against reinoculation with severe *X*. Over a month after infection, however, a few yellow rings appeared on the reinoculated and non-inoculated plants alike. On *Datura* there was a whitish semi-necrotic etching of the veins, first observed 16 days after inoculation, but the systemic veinal mottle described under 6(*a*) did not persist. Inoculated *Capsicum* plants exhibited local lesions followed by severe systemic necrosis. Epicure (two plants) was unaffected by grafts from Chuhuavisca, though the scions made good unions and grew vigorously to a height of 2 ft. Grafts from the original potato to President resulted in a spotty mottle on the stock with scattered superficial necroses such as is sometimes seen with Up-to-Date streak on this host and may best be regarded as an arrested form of top necrosis. U.S.D.A. 41956 remained apparently healthy when grafted with Chuhuavisca.

Both the above viruses are clearly related to the *X* group, as shown by their complete protection against reinoculation with severe *X*, but they differ from all known strains of that virus in failing to cause top necrosis in Epicure.

(*c*) *Chohuahineca*. This plant, which showed no sign of disease, contained a virus differing from all the potato viruses known in this country in being readily sap-transmissible to *Datura* and not to tobacco. Tobacco plants inoculated from Chohuahineca remained perfectly healthy, nor could any virus be recovered from them causing symptoms on *Datura*.

Direct sap inoculation from Chohuahineca to *Datura* resulted in formation of a few round brown local lesions 2–3 mm. across, first observed 12 days after inoculation (Pl. VI, fig. 3). Systemic symptoms were not seen until 1 month after inoculation, when a mild systemic interveinal mottle developed, which became brighter yellow green with a few yellow spots but was not associated with rufflling or deformity of the leaves. When the local lesions were punched out and inoculated to young *Datura* plants fresh local lesions resulted, followed by much more severe interveinal mottle with small necroses, deformity of the leaves and stunting of the plants (Pl. VI, fig. 4). It seems, therefore, that a mixture of virus strains was present and that the most severe as regards *Datura* could be selected by inoculating only from the local lesions. Inoculation to *Nicotiana glutinosa* induced a mild systemic interveinal mottle with no local lesions. No symptoms resulted from inoculation to tomato, nor could the virus be recovered from this host. Inoculation to *Capsicum* gave no local lesions but a mild systemic interveinal mottle with small interveinal necroses. Inoculation to *Lycium barbarum* led to negative results or to the formation of faint local rings only.

Grafting of Chohuahineca to Epicure induced no disease in the stock, while in a similarly treated King Edward only a bright interveinal mottle was produced, with some rugosity and waving of the leaves (Pl. VI, fig. 2). From Epicure the virus was recovered unchanged on *Datura*. Scions from a second plant of Chohuahineca killed Epicure, President, President carrying mild *X*, Sharpe's Express and British Queen with top necrosis. This plant probably therefore contained a strain of *X* as well as the streak virus. Grafted plants of Arran Victory and Up-to-Date remained healthy, although the presence of the virus in the former was confirmed by grafting back to President which then developed top necrosis. Similar grafts from Chohuahineca resulted in blotchy veinal mottle with slight welling and waving of the leaves on Katahdin (Pl. VI, fig. 1) and in veinal necrosis followed by severe chlorosis and leaf drop on Eclipse. A grafted plant of U.S.D.A. 41956 developed a bright veinal mottle with no necrosis but a dropping of the lower leaves. Grafts from this plant to President led to top necrosis of the latter, but neither local nor systemic symptoms resulted from sap inoculations to Arran Crest, Arran Pilot, Arran

Victory, Doon Early, Doon Star, Dunbar Yeoman, Katahdin, President, Up-to-Date or U.S.D.A. 41956. Inoculation from the same plant to *Datura*, however, gave local lesions and systemic interveinal mottle as described above, while no reaction followed inoculation to *Capsicum*. This *X*-free stock of the Chohuahineca virus was used in a study of its physical properties in *Datura* sap with the following results. The virus was recovered unchanged after heating for 10 min. at 60° C. but not at 65° C. It was recovered on six out of six *Datura* plants after dilution to 1 in 10,000, on two out of six after dilution to 1 in 100,000 and on none out of six after dilution to 1 in 1,000,000. It survived at least 5 days ageing *in vitro*.

This virus bears considerable resemblance to Up-to-Date streak, virus *B*, but differs from it in not infecting tobacco and tomato and in its effect on certain potato varieties. Its reactions are compared with those of *B* and *C* in Table II where + indicates top necrosis (cf. Bawden, 1936).

Table II. *Reactions of Chohuahineca virus, and viruses B and C on certain potato varieties*

	Epicure	President	Arran Victory	Up-to-Date	British Queen	Katahdin	U.S.D.A. 41956
Chohuahineca	−	+	−	−	+	−	−
B	+	+	+	−	+	+	−
C	?	+	−	+	+	?	+

The variety Chupica yarama apparently contains a similar virus.

(*d*) Two other varieties, Cochicallo and Lanta mari, both infected with a mild *X*, also contained streak viruses which were not fully investigated. That of the former killed Arran Victory with top necrosis, induced bright yellow interveinal mottle on British Queen, and gave no visible reaction on U.S.D.A. 41956, Epicure, President or Up-to-Date. That of the latter killed Dunbar Yeoman with top necrosis but had no visible effect on President.

(7) *Leaf roll.* None of the Peruvian varieties showed typical symptoms of leaf roll early in the season, but a number exhibited a rolling of the lower and intermediate leaves as they approached maturity. These were Azul parcco, Hanco cuillo, Huaca huajra and Parcco caramo. In the case of Huaca huajra the symptoms of leaf roll developed within 10 weeks after emergence of the plant above ground. It was then 30 in. high with the lower leaves harsh in texture, much rolled with interveinal pallor and some anthocyanin development on the lower surfaces. Grafts to British Queen, Majestic and President failed to transmit leaf roll as the stocks

died of top necrosis, but in the case of a graft to Epicure one shoot of the stock died of top necrosis while two others developed symptoms resembling leaf roll.

A graft from Parcco caramo to President induced slight interveinal pallor and very slight anthocyanin development on the lower leaves, but full symptoms of leaf roll did not appear.

In the case of Yana imilla one plant which exhibited a bright yellow Aucuba-like mottle on most leaves apparently contained viruses $X+B$ (see (4), p. 94). Another plant, which appeared healthy, when grafted to President induced typical symptoms of leaf roll on some lower leaves; these were followed by a long growth of healthy shoots, until some 4 months after grafting the upper leaves developed interveinal pallor in patches towards the margin with traces of anthocyanin on the lower surface. Rolling of leaves also resulted in a plant of Dunbar Cavalier grafted from the same source.

Chachaza, which only showed slight interveinal mottle and no symptoms of leaf roll, also induced interveinal pallor, rolling, pointing and anthocyanin development in a plant of President potato, some 5 weeks after grafting. Later, however, the new growth of stock developed in a normal manner, though the lower leaves remained rolled.

The evidence of presence of leaf roll is thus not conclusive but it seems likely that strains of this or a similar virus occurred in the varieties mentioned above.

(8) *Viruses A and Y.* No evidence has been obtained of the presence of either of these viruses. All plants whose appearance at all suggested crinkle were grafted to Up-to-Date or British Queen, but the stocks either remained healthy or, if top necrosis supervened, there was clear evidence from other sources of the presence of a streak virus capable of bringing about such a reaction.

All plants suspected of infection with *Y*, in addition to the routine test on tobacco, which should have detected that virus, were inoculated to seedlings of *Lycium barbarum*, lately shown to be a useful diagnostic host for *Y* (Dennis, 1938).

IV. Infection of healthy Peruvian potatoes with European viruses

A small amount of material of the varieties listed in (1), p. 92, was available for infection experiments designed to explore their reactions to European viruses. The results may be summarized briefly as follows:

(1) *Ccompis.* Developed typical leaf roll when grafted from infected British Queen. Developed interveinal mottle when grafted with President carrying a mild *X*.

(2) *Ccosi.* Developed typical leaf roll when grafted from infected British Queen. Carried *X* when grafted with that virus in Great Scot. The virus was recovered from Ccosi by inoculation to *Datura*.

(3) *Milagro.* Succumbed to top necrosis when grafted with Di Vernon carrying *X*+*C*. Remained healthy when sap-inoculated with mild *X* alone.

(4) *Phospa sunchus.* Developed typical leaf roll when grafted with infected British Queen. Remained apparently healthy when grafted with Di Vernon carrying *X*+*C*, but scions taken from it to President killed the stock with top necrosis. Developed typical veinal mottle, rugosity and leaf drop when grafted with *Y* in Bintje.

(5) *Sacampai choque.* Succumbed to top necrosis when inoculated with mild *X*. Developed only veinal mottle with neither necrosis nor leaf drop when grafted with *Y* in Bintje.

Tubers of a small number of varieties, which remained after all other requirements had been met, were planted in the field and exposed to natural infection by aphids. Under these conditions plants of the following varieties developed symptoms of leaf-drop streak: Azul parceo, Cacho amajaya, Ccosi, Chinamajaya, Huaca huajra, Huaca nuño, Phospa sunchus, Pinta milagro.

V. Summary

Exposure of the Peruvian potato varieties to full summer-day conditions at Cambridge resulted in a more uniform growing period, and increased the tendency to bloom. The effect on tuber production varied according to the variety. In most types the weight and number of tubers were not adversely influenced, but there was a tendency to elimination of the dormancy period and to conversion of the stolons into aerial shoots. In a few varieties there was no adverse influence, and in the members of one small group plants exposed to full-day conditions formed no tubers.

Investigation of virus content showed that of fifty-nine varieties only eleven were healthy. In the remaining forty-eight varieties there were found viruses apparently identical with those known in this country as *X*, *B*, *C*, *F*, *G* and perhaps leaf roll.[1] There was also evidence of the presence of other viruses, suggesting the existence in South America of a virus

[1] Equivalent respectively to the *Solanum* viruses 1, 4, 5, 8, 9 and 14 of Dr K. M. Smith's classification. *Solanum* virus 6 of the same classification is the foliar necrosis strain of *X* (see p. 92).

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DENNIS.—Notes on the Photoperiodic Reactions and Virus Contents of some Peruvian Potatoes (pp. 87–101)

complex, only isolated members of which have hitherto been known in Europe. It follows that great caution should be exercised in introducing South American varieties into potato-growing districts.

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REFERENCES

Bawden, F. C. (1934). Studies on a virus causing foliar necrosis of the potato. *Proc. roy. Soc.* B, **116**, 375–95.

—— (1936). The viruses causing top necrosis (acronecrosis) of the potato. *Ann. appl. Biol.* **23**, 487–97.

Bukasov, S. M. (1933). *The Potatoes of South America and their Breeding Possibilities.* Leningrad.

Clinch, P. E. M., Loughnane, J. B. & Murphy, P. A. (1936). A study of the aucuba or yellow mosaics of the potato. *Sci. Proc. R. Dublin Soc.* N.S. **21**, 431–48.

Dennis, R. W. G. (1938). A new test plant for potato virus *Y*. *Nature, Lond.*, **142**, 154.

Garner, W. W. & Allard, H. A. (1920). Effect of length of day and other factors of the environment on growth and reproduction in plants. *J. agric. Res.* **18**, 553–606.

—— —— (1923). Further studies in photo-periodism. The response of the plant to relative length of day and night. *J. agric. Res.* **23**, 871–920.

Salaman, R. N. (1933). Protective inoculation against a plant virus. *Nature, Lond.*, **131**, 468.

Tincker, M. A. H. (1925). The effect of length of day upon the growth and reproduction of some economic plants. *Ann. Bot., Lond.*, **39**, 721–54.

EXPLANATION OF PLATES V AND VI

PLATE V

Fig. 1. Interveinal mottle on President potato induced by the strain of *X* obtained from Alpaca noza.

Fig. 2. Top necrosis of U.S.D.A. 41956 following a graft from Parcco hancco.

Fig. 3. Necrotic rings on Arran Victory leaf caused by systemic infection with the Parcco hancco virus—? virus *C*.

Fig. 4. Tuber of Cacho amajaya sprouted before harvesting.

Fig. 5. *Datura stramonium* infected with the virus recovered from Papa runtusa.

PLATE VI

Fig. 1. Veinal mottle and ruffling of Katahdin leaf due to systemic infection with the Chohuahineca virus.

Fig. 2. Top of a King Edward potato infected with the Chohuahineca virus.

Fig. 3. Chohuahineca virus on *Datura stramonium*: local lesions.

Fig. 4. Chohuahineca virus on *Datura stramonium*: systemic infection.

Fig. 5. Faint local lesions of the strain of *X* recovered on *Datura stramonium* from Alcea fina.

Fig. 6. Leaf of Luqui mari showing characteristic small necroses.

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THE INTRACELLULAR INCLUSIONS OF SOME PLANT VIRUS DISEASES

By F. C. BAWDEN and F. M. L. SHEFFIELD

Rothamsted Experimental Station, Harpenden, Herts

(With Plates VII and VIII)

CONTENTS

Introduction

Although intracellular inclusions have not been found in all virus diseases, they seem to be specific to these diseases and, in some, they are sufficiently frequent and characteristic to be of diagnostic value. Their production depends more on the infecting virus than on the species of host. For example, they have been found in a number of different species infected with tobacco mosaic virus, but not in any of the same species infected with potato virus Y or cucumber virus 1. It is not doubted that they are formed as a result of virus infection, but different workers have held widely divergent views as to their nature and significance. Some have regarded them as amoebae or as stages in the life history of a causative organism, to which names have occasionally been given, while others have regarded them simply as masses of coagulated cytoplasm.

With animal viruses it is more generally believed that the inclusion bodies are aggregates of virus particles. The use of ultra-violet light and microscopes of high resolving power has shown that the inclusions accompanying infection with some of the larger viruses contain numbers of elementary bodies with approximately the same size as that estimated for the virus particles by filtration experiments. However, no adequate reason has yet been advanced to explain the aggregation of virus particles

which form stable suspensions *in vitro* into such characteristic bodies in the infected cells.

It has been found that plants infected with certain viruses contain proteins not present in healthy plants. These proteins have the characteristic properties associated with the different viruses, changes in them result in loss of infectivity, and it is highly probable that they are the viruses themselves. Beale (1937) has pointed out some features that one type of inclusion has in common with the protein isolated by Stanley (1936) from plants infected with tobacco mosaic virus. In this paper further similarities and differences between the behaviour of the inclusions and the purified virus preparations are described.

The normal cell

Since a number of cell constituents have been described by various workers as possible causes of virus diseases or as virus inclusion bodies, a brief account of a normal adult solanaceous cell is given. The walls of parenchymatous cells are reinforced with cellulose and are birefringent. The thickness of the cellulose layer varies in different tissues, being thin in pith and cortical cells, and thick and reinforced with cutin in the outer walls of the epidermal cells and in the hairs. The cell contains cytoplasm and nucleus with cell sap occupying the central vacuole. The cytoplasm forms a continuous layer inside the wall, and strands cross the vacuole. It streams continuously around the cell, its movement being obvious because of the chondriosomes, plastids, oil globules and other particles embedded in it. The nucleus, an oval or spherical body containing one or more readily distinguished nucleoli, is also embedded in and carried round by the cytoplasm. Indications of the chromatin reticulum can sometimes be seen, but this is more obvious after fixing and staining. Most cells contain only one nucleus, but occasionally, e.g. in some adult pith cells, two are found. Sometimes, as in the formation of necrotic lesions in *Nicotiana glutinosa*, the binucleate condition is a result of virus infection.

In addition to the substances in solution, the sap and cytoplasm contain a number of crystals, globules and particles (Pl. VII, figs. 1, 2). The commonest crystals appear to be octahedra; these are inorganic, readily dissolve in hydrochloric acid, and are probably calcium oxalate. They vary in size and occur most frequently in the epidermis over the veins, where from one to a dozen or more can usually be found in a single cell. Globules, giving the staining reactions of both oils and proteins, occur in some plants, especially in the younger tissues (Sheffield, 1933),

and alkaloids occur in solution. Of most interest and frequent occurrence are particles, many of which superficially resemble small bacteria, varying in length from about 3μ down to limits of resolution. They can be found in large numbers in almost all cells and show active Brownian movement, but no autonomous movement. They vary in shape and perhaps in chemical composition, but they are too small for successful microchemical examination; some are definitely rod-shaped, while others are elliptical and a few are angular plates. When the cell dies they aggregate, and the addition of fixatives also often causes them to clump together. The larger of the particles are definitely birefringent with straight extinction, and when the cell is examined between crossed Nicol prisms they repeatedly appear and disappear as Brownian movement alters their position to the plane of polarization. It is possible that these particles are similar to the larger birefringent bodies described by Schmitt & Johnson (1938) in the microspores of *Tradescantia*, which they suggest are storage proteins. Bacteria often occur on the surface of hairs and epidermal cells, but we have never seen any in the cells of healthy or mottled plants. As these particles more nearly resemble bacteria than any of the other cell constituents it is possible that they are the short rod-shaped bacteria described by Iwanowski (1903) which formed apparent zooglea when fixed.

Cells infected with strains of tobacco mosaic virus

Except in plants such as *Nicotiana glutinosa* which react to infection by the formation of necroses and have their cell contents destroyed, all the constituents described in healthy cells can also be found in infected cells, although the amount of cytoplasm often seems to be reduced considerably. The microscopic appearance of infected cells varies with the external symptoms, and depends on the strain of the virus and on the age of the tissues. If old leaves are infected, or a masked strain of the virus used, there are no external symptoms nor any definite microscopic changes in the cells. In mottled leaves some hypoplasia or hyperplasia occurs and abnormalities of the chloroplasts can be seen. Cytoplasmic streaming often seems to be increased early in infection, and greater numbers of the crystals and particles found in healthy plants may be seen.

The greatest difference between the healthy and infected cell is the formation of the characteristic inclusions in the latter. As early as 1903 Iwanowski described two main types in tobacco mosaic plants, one consisting of amorphous material, later named *X*-bodies by Goldstein (1924), and the other flat crystalline plates. The three strains of tobacco

mosaic virus that we have used mainly, those causing common tobacco mosaic, Enation mosaic and Aucuba mosaic, all cause the formation of the two kinds of inclusion. No significant differences have been found between the crystalline inclusions produced by the three strains (Pl. VII, figs. 3*a*, 4*a*; Pl. VIII, fig. 2), but the amorphous body of Aucuba mosaic differs in some respects from those of tobacco mosaic and Enation mosaic.

The amorphous[1] bodies of all three diseases are relatively stable entities, are preserved by ordinary cytological fixatives and give all the usual protein reactions. Those of tobacco mosaic and Enation mosaic, diseases with closely similar external symptoms, are small and superficially resemble amoebae. They average about 10μ in length, contain vacuoles, chondriosomes and oil globules, and as they are carried round the cell by the streaming cytoplasm frequently change their shape. The amorphous body of Aucuba mosaic, a disease readily distinguished from the other two, is larger, somewhat more granular and less like the surrounding cytoplasm. In some hosts the body is rather diffuse, but in others, especially *Solanum nodiflorum*, it may be spherical with a diameter of 30μ in large cells. There is no reason to assume that there is any essential difference between the amorphous bodies produced by the three virus strains, but we have been unable to follow the method of formation and disappearance of the amoeboid *X*-bodies as has been done in detail with the bodies of Aucuba mosaic (Sheffield, 1934).

At the time that external symptoms of Aucuba mosaic become obvious, minute particles containing protein appear in the circulating cytoplasm. These are carried round the cell and on coming together fuse. The aggregation continues until all the particles are joined together into one body. The outside layer will be subjected to rather different surface tension forces from the material inside the body, and this may be responsible for the properties of the body suggesting an external membrane (Goldstein, 1926; Sheffield, 1939). After some time the body degenerates and its place is taken by crystalline plates.

Over periods of some years the type and relative proportions of different kinds of inclusions produced by Aucuba mosaic virus have altered appreciably. Some years ago in *S. nodiflorum* amorphous bodies were produced in large numbers early in infection, and crystalline plates appeared to be formed only from disintegrating bodies. Crystal-like spikes, often as long as the cell, were also frequently seen (Henderson Smith, 1930; Sheffield, 1931). Recently we have found no spikes and fewer

[1] The term "amorphous" in describing inclusions is used as opposed to "crystalline".

amorphous bodies, but large numbers of crystals. Even when plants were infected in 1936 with virus from leaves dried in 1927 no spikes were found, but these plants contained large numbers of amorphous bodies. After this source of the virus had been subcultured continually for two years it also produced large amounts of crystalline material without first forming the bodies.

The crystalline plates may occur in large numbers either alone or in the same cells as the amorphous bodies. They are best examined in fresh living cells, because fixation destroys them or causes the production of numerous striations, an effect responsible for the name "striate material" given to this type of inclusion. Like the amorphous bodies the plates give protein reactions, but they are extremely fragile and either disappear or alter in appearance if the cell is injured in any way. They are colourless and transparent, and have a refractive index higher than the cell sap. They are true crystals with a three-dimensional regularity, and as they slowly move around the cell and turn over they show both side and end faces. Faint striations can often be seen in the side faces of untreated crystals, especially if they are examined in polarized light. When seen in the basal plane some plates are regular hexagons, with all their angles 120° (Pl. VIII, fig. 2). More often they are irregular with only an occasional angle, suggesting that they are formed by unequal growth or by the fusion of separate crystals. Early in infection several crystals can often be found in one cell, but later they coalesce into a single, rather shapeless mass.

When seen edgeways they are oblong, and between crossed Nicol prisms are birefringent with straight extinction. They are not birefringent when examined flat (Pl. VII, figs. 3*a*–4*b*). The refractive index is greater along the thickness than along the length of the crystals, and as some are definitely hexagonal it is highly probable that they are hexagonal crystals. However, this could not be confirmed, since we were unable to obtain an interference figure, probably because of the small size of the crystals and because of the presence of the birefringent cell wall which greatly affects a critical examination in polarized light.

The characteristic inclusions cannot be found at all times in infected plants. In good growing conditions the first microscopic changes can be seen a week or so after infection when external symptoms become evident in the young leaves. The formation of the amorphous bodies of Aucuba mosaic commences at this time, and a few *X*-bodies and a little crystalline material can be found in plants with Enation or tobacco mosaic. The inclusions rapidly increase in number, reaching a maximum about a

month after infection. After a further month the amorphous bodies of Aucuba mosaic disintegrate and form crystals, and those of tobacco mosaic disappear. The crystalline inclusions persist for longer than the amorphous bodies, but after a few months both types have usually disappeared.

Comparison of the intracellular inclusions with purified preparations of the strains of tobacco mosaic virus

Purified preparations of tobacco mosaic type viruses have features in common with crystalline materials. They give an X-ray pattern and are birefringent (Wyckoff & Corey, 1936; Bawden *et al.* 1936), but no visible preparations have yet been made *in vitro* which are true crystals. The particles in the purified preparations are greatly elongated and, therefore, readily orientated. Solutions have a characteristic sheen; when dilute they show the phenomenon of anisotropy of flow strongly, and when concentrated they are liquid crystalline. Increasing the solid content by evaporation or by high-speed centrifugation causes the formation of anisotropic gels (Bawden & Pirie, 1937*a*). Precipitating the virus with acid or ammonium sulphate greatly increases the sheen and produces birefringent needle-shaped bodies. These are fibrous in appearance, have pointed ends and no facets, but have been called crystals (Stanley, 1937). Bernal & Fankuchen (1937) have shown that the constituent particles in the needles are arranged parallel one to the other without any regularity of arrangement in the direction of their length. They are therefore not true crystals, but are a form of liquid crystal and are more accurately called paracrystals or microtactoids.

Iwanowski (1903) and Goldstein (1924) found that the addition of acid to the crystalline intracellular inclusions caused them to break down into needles. Beale (1937) has pointed out that in appearance these needles closely resemble the paracrystals of the purified virus. She also found that the *p*H stability range of the needles in the cells was approximately that of the virus *in vitro*, and it seems highly probable that the crystalline inclusions are rich in virus.

Purified tobacco mosaic virus is miscible with water in all proportions and does not settle out in a solid form when solutions are concentrated by evaporation. The fluids become increasingly viscous, and when the solid content is sufficiently great they turn to gels. A solid, or quasi-solid phase, the paracrystals, separates from solutions only if acid is added to reduce the *p*H to below 4 or if much salt (1/5th saturation with ammonium sulphate) is added. As the virus content of fully infective sap is only

about 0·3%, the *p*H of cell sap is above 6, and the salt content is too small to precipitate the virus, it is highly improbable that the crystalline inclusions are deposits of pure virus, for there is no apparent reason for pure virus to settle out in these conditions. It is perhaps more probable that they are composed of an insoluble complex, formed by the union of the virus with some constituent or constituents of the host.

The purified virus readily unites with some protamines and histones, the one most studied being clupein (Bawden & Pirie, 1937*a*, *b*), to form complexes whose behaviour in many ways resembles that of the crystalline inclusions. They are insoluble in dilute salt solutions near neutrality, conditions probably obtaining in the cells of young plants with a high water content. When a neutral solution of clupein sulphate is added to preparations of any of the strains of tobacco mosaic virus a precipitate with a pronounced sheen immediately separates. The precipitated material consists of fibres which microscopically closely resemble the paracrystals produced with acid or strong salt solutions, but some are longer and appear to be similar to the mesomorphic fibres which Best (1937) described as settling out from samples of clarified infective sap after keeping for some months (Pl. VIII, fig. 1). The insoluble complexes contain less than 5% of clupein, apparently too little to affect the orientation of the particles. Their solubility varies greatly with small changes in *p*H and salt content, and also depends on the virus strain and on the relative amounts of virus and clupein in the system. The complexes all dissolve in salt solutions more concentrated than *N*/10, but the minimum amount of salt required for solution varies with the *p*H and is more at *p*H 5·5 than at *p*H 6. The stability of these precipitates is therefore similar to that of the crystalline inclusions which either dissolve or break down into needles if the cell is injured or has its *p*H altered by the addition of acid. If the crystalline inclusions are complexes of this type their formation in young tissues and their disappearance in plants infected for long periods can be explained on the basis of changes in *p*H and salt content which occur with increasing age. The sap of young plants has a relatively high *p*H value and low salt content. In these conditions such complexes would be insoluble and readily settle out, whereas with increasing acidity and salt content they would become increasingly soluble and tend to disappear.

There is one important difference between the artificial complexes produced with the purified viruses and the inclusions formed in the plants, for the former are paracrystalline whereas the inclusions are true crystals. This may merely mean that correct conditions for the production

of true crystals *in vitro* have not yet been found. However, at the present time it seems that the apparent difference in the behaviour of the virus in the plant and after purification is more probably a result of changes in the physical state of the virus brought about by the processes of purification. Precipitation of the virus in sap from young infected plants with acid or salts and resolution causes a reduction in the filterability and infectivity, and an increase in the anisotropy of flow. These results suggest that precipitation has increased the size of the infecting units by causing the virus particles to aggregate linearly (Bawden & Pirie, 1937*a*). It seems that the virus particles as first formed in the plant are relatively small, and when rendered insoluble can arrange themselves regularly in three dimensions to form true crystals, but that the small particles readily come together to form greatly elongated micelles in which state they produce liquid crystals.

As precipitation of the virus *in vitro* has such a definite effect on its physical properties, if the crystalline inclusions are largely composed of virus it might be expected that precipitation in the plant would also have some effect. There is now a good deal of evidence that virus units of different sizes do occur in clarified infective sap. Wyckoff (1937) finds only one sedimentation constant (174×10^{-13} cm. sec.$^{-1}$ dynes^{-1}) for the virus from plants infected for short periods, but sap from plants infected for a month, or sap treated chemically, readily gives a second larger component with a sedimentation constant of 200×10^{-13}. A similar effect is also found in the optical properties of clarified infective sap. Sap from plants infected for short periods shows little or no anisotropy of flow when shaken between crossed polaroid plates unless the virus is first precipitated with acid or ammonium sulphate. With increasing length of infection period the amount of anisotropy of flow increases, and clarified sap from plants infected for a few months shows the phenomenon strongly, and little increase is obtained by precipitating and redissolving the virus. The larger virus particles therefore do not seem to occur until the crystalline inclusions are formed, and the proportion of these particles present in sap increases with the formation and resolution of the inclusions. The filtration results of Smith & MacClement (1938) also supply further evidence for particles of different sizes in infective sap, for they find that tobacco mosaic virus at different times has filtration end-points of 50 and 180 mμ. However, as the conditions determining the end-point are not given, these results cannot be correlated with the formation of crystalline inclusions.

The amorphous inclusion body is at first sight more difficult to connect

directly with the virus, but the formation of crystals, indistinguishable in appearance and behaviour from the crystalline inclusions, within degenerating bodies of Aucuba mosaic, suggests that the two types of inclusion have a common constituent. Also, Sheffield (1939) has shown that the isolated and carefully washed bodies from Aucuba mosaic are infective, and they therefore contain virus. As they also contain chondriosomes and oil globules, this cannot be taken as proof that the virus is an essential part of the body for it may have been adsorbed during the formation and circulation of the body around the cell. However, as the estimated weight of such inclusions is little greater than the minimum weight of purified virus necessary to cause infection, it seems that their infectivity is too great to be explained merely by the adsorption of virus. If these bodies are insoluble virus-host complexes, their amorphous nature, greater stability and different solubility indicate that the virus is combined with a constituent of the host different from that suggested for the crystalline inclusions, and that the ratio of this constituent to virus is greater than in the crystalline inclusions.

Insoluble virus-protein complexes, probably containing less than 50 % of virus, and superficially resembling the inclusion bodies, are produced when preparations of strains of tobacco mosaic virus are mixed with their antisera. Amorphous precipitates settle out which are much more stable than the paracrystalline precipitates produced by clupein, and which are unaffected by the addition of acid and are insoluble in salt solutions. If the virus-antiserum mixtures are kept circulating by convection currents the sequence obtained also closely resembles that of the formation of the body of Aucuba mosaic. As soon as the antiserum and virus have become mixed small particles appear throughout the fluid. In moving around these come into contact and fuse together until, finally, all the precipitate settles out in a fluffy mass. The precipitate is only formed if antibody and antiserum are present in correct proportions and its formation is greatly inhibited by the presence of too much virus. Also, although the solubility of virus-antibody complex is unaffected by small changes in *p*H or salt content, the precipitate dissolves fairly readily if excess virus is added. If the amorphous inclusions are virus complexes with solubility relationships similar to those formed by the union of virus and antibody, their formation early in infection, when the virus content of sap is low, and their disappearance later with increasing virus content could be explained.

It is not intended to suggest that the amorphous inclusions are produced by a union of virus and antibody in the plant, for there is no valid

evidence that plants contain or produce antibodies, and the analogy is put forward only to show that the apparent behaviour of the virus in the plant can be closely simulated *in vitro*. However, it is possible that the formation of inclusions does in part act as a protective mechanism, for it is difficult to imagine any virus that may be rendered insoluble in the inclusions possessing great biological activity.

Because of its greater stability the amorphous inclusion is more suitable for microchemical tests than the crystalline inclusion. It gives all the protein reactions of the purified virus, but there is no critical chemical test for detecting small amounts of the virus. Bawden & Pirie (1937*a*) have shown strains of tobacco mosaic virus to be nucleoproteins, differing from the nucleoproteins characteristic of nuclei in that the nucleic acid contains ribose instead of a desoxy pentose. Feulgen's reagent readily identified desoxy pentose, but, unfortunately, there is no simple colour test for detecting nucleic acids of the ribose type. The amorphous body does not contain a desoxy pentose, and staining with Feulgen's reagent sharply distinguishes it from the nucleus, for the body is unaffected whereas the nucleus takes on a deep red or purple colour.

It is generally accepted that virus-infected plants contain intracellular inclusions only when they are showing external symptoms, and in plants infected with a masked strain of tobacco mosaic virus Beale (1937) found inclusions only in the occasional chlorotic areas. She suggests that the formation of inclusions is dependent on the concentration of the virus, and that only in the chlorotic areas is there sufficient virus for it to crystallize. Although this suggestion cannot be disproved there is some evidence against it. Merely increasing the virus content of purified preparations, even to twenty or more times that of infective sap, does not cause the virus to settle out in solid form. Also, the inclusions tend to disappear late in infection when the virus content of sap is greatest, and the virus content of plants fully infected with the masked strain is probably greater than that of plants with tobacco mosaic when they first commence to form inclusions. If the inclusions are complexes of the type we have suggested their formation will depend on the presence in infected plants of materials which can unite with the virus and render it insoluble. The close association of inclusions with external symptoms might then be determined, not by the greater virus content of obviously diseased plants but by the fact that only these plants contain such materials. In other words, the substances with which it is suggested that the virus combines may not be normal constituents of cells but disease products, the presence of which is indicated by external symptoms.

Infections with viruses other than tobacco mosaic virus

In addition to the strains of tobacco mosaic virus we have also worked with tobacco ring-spot virus, potato viruses *X* and *Y*, tomato Bushy stunt virus, Hyoscyamus virus 3, and cucumber viruses 1, 3 and 4. We have not found intracellular inclusions in plants infected with potato virus *Y* or with any of the cucumber viruses, and if they occur it must be either in different conditions or with much less frequency than with the others. Their apparent failure to produce inclusions might of course be a result either of differences between them and the other viruses or of differences in their host plants. With potato virus *Y* and cucumber virus 1 it cannot be attributed to an effect of the host plants, for they infect a number of solanaceous species in which other viruses produce numerous inclusions. Nothing is known of the chemical nature of these two viruses or of the conditions causing them to precipitate, and, at the present time, their different behaviour in the plant cannot be correlated with any properties of the viruses *in vitro*.

It seems more probable with cucumber viruses 3 and 4 that the absence of inclusions may be an effect of the host plant. No chemical differences have been found between these viruses and tobacco mosaic virus, and only slight differences in their physical properties. They have antigens in common with tobacco mosaic virus, and *in vitro* precipitate in the same liquid crystalline forms and under closely similar conditions (Bawden & Pirie, 1937*b*). Only members of the Cucurbitaceae have been infected with cucumber viruses 3 and 4, and as these are immune to tobacco mosaic virus there are no common hosts known in which the behaviour of the viruses can be compared. It is possible that cucumber plants do not contain materials capable of uniting with and precipitating the viruses, or alternatively, if complexes are formed, cucumber sap is so much more alkaline than tobacco sap that complexes insoluble in tobacco might be soluble in cucumber.

Results with tobacco ring-spot virus show that the host can determine the formation of inclusions and that cucumber is not a favourable host. In tobacco and other solanaceous plants this virus causes the production of large numbers of inclusions, the majority being amorphous and of the amoeboid form similar to those produced by tobacco mosaic virus. In addition, crystalline inclusions are produced; these are colourless and hyaline like those caused by tobacco mosaic virus, but they occur frequently (Pl. VIII, fig. 3). They also appear to have a different crystal form, for no hexagonal plates have been seen, the majority being

rectangular blocks which are birefringent when viewed along all axes. Infected cucumbers show severe symptoms, but of a different type from tobaccos. We have found no crystalline inclusions in infected cucumbers and only a few amorphous bodies. These are formed very infrequently and only in certain leaves, although the whole plants are severely diseased.

Potato virus *X* is a nucleoprotein forming liquid crystalline solutions and gels similar to those of tobacco mosaic virus, but when precipitated with acids, salts or clupein it is amorphous and not paracrystalline (Bawden & Pirie, 1938*a*). In keeping with this behaviour *in vitro* we have found only amorphous inclusion bodies in plants infected with this virus, although Clinch (1932) states that in one plant she found striate material. The inclusions are very similar to the *X*-bodies of tobacco mosaic, but differ from them in that they occur chiefly in the assimilatory tissues instead of in the hairs and epidermis.

Plants infected with Hyoscyamus virus 3 contain large numbers of amorphous inclusions in all tissues. These resemble the bodies of Aucuba mosaic both in appearance and in their manner of formation. We have found no crystalline inclusions, but when the bodies disintegrate they give rise to numerous long, very thin needles (Pl. VIII, fig. 4). These show no extinctions in a polarizing microscope, but whether this is because of their small size or because they are not birefringent is not known. Little is known about the chemical and physical properties of this virus; we have obtained preparations showing anisotropy of flow, but the precipitates from these with strong salt solutions were amorphous.

Tomato bushy stunt virus has also been shown to be a nucleoprotein, but to differ in many ways from the other viruses that have been isolated. Solutions are isotropic and, when precipitated with salts or with clupein sulphate, the purified preparations crystallize in the form of rhombic dodecahedra (Bawden & Pirie, 1938*b*). In many plants suffering from bushy stunt we have been unable to find any definite inclusions. In a few we have found amorphous bodies of the tobacco mosaic type (Pl. VIII, fig. 5) and all contain much crystalline material. Some of the crystals appear to be dodecahedra and have not been seen in healthy plants. However, as the crystals and birefringent particles of uninfected cells often occur in greatly increased numbers and the apparent dodecahedra are found only occasionally, it is impossible to be sure that these are true virus inclusions.

SUMMARY

The contents of healthy cells and those infected with a number of different plant viruses are described. Some of these viruses apparently do not cause the production of intracellular inclusions; others cause the production of amorphous bodies only, and the remainder produce both amorphous and crystalline inclusions. The properties of the inclusions are compared with those of purified preparations of the viruses. It is shown that insoluble complexes of the viruses with protamines, histones and proteins which in many ways resemble the intracellular inclusions can be produced *in vitro*. Possible explanations for the formation and disappearance of the inclusions in infected plants are suggested.

REFERENCES

BAWDEN, F. C. & PIRIE, N. W. (1937*a*). *Proc. roy. Soc.* B, **123**, 274.
—— —— (1937*b*). *Brit. J. exp. Path.* **18**, 275.
—— —— (1938*a*). *Brit. J. exp. Path.* **19**, 66.
—— —— (1938*b*). *Brit. J. exp. Path.* **19**, 251.
BAWDEN, F. C., PIRIE, N. W., BERNAL, J. D. & FANKUCHEN, I. (1936). *Nature, Lond.*, **138**, 1051.
BEALE, H. P. (1937). *Contr. Boyce Thompson Inst.* **8**, 413.
BERNAL, J. D. & FANKUCHEN, I. (1937). *Nature, Lond.*, **139**, 923.
BEST, R. J. (1937). *Nature, Lond.*, **139**, 628.
CLINCH, P. (1932). *Proc. R. Dublin Soc.* **20**, 143.
GOLDSTEIN, B. (1924). *Bull. Torrey Bot. Cl.* **51**, 261.
—— (1926). *Bull. Torrey Bot. Cl.* **53**, 499.
IWANOWSKI, D. (1903). *Z. PflKrankh.* **13**, 1.
SCHMITT, F. O. & JOHNSON, G. T. (1938). *Ann. Mo. bot. Gdn*, **25**, 455.
SHEFFIELD, F. M. L. (1931). *Ann. appl. Biol.* **18**, 471.
—— (1933). *Ann. appl. Biol.* **20**, 57.
—— (1934). *Ann. appl. Biol.* **21**, 430.
—— (1939). *Proc. roy. Soc.* B. In the press.
SMITH, J. H. (1930). *Ann. appl. Biol.* **17**, 213.
SMITH, K. M. & MACCLEMENT, W. D. (1938). *Proc. roy. Soc.* B, **125**, 291.
STANLEY, W. M. (1936). *Phytopathology*, **26**, 305.
—— (1937). *Amer. J. Bot.* **24**, 59.
WYCKOFF, R. W. G. (1937). *J. biol. Chem.* **121**, 219.
WYCKOFF, R. W. G. & COREY, R. B. (1936). *J. biol. Chem.* **116**, 51.

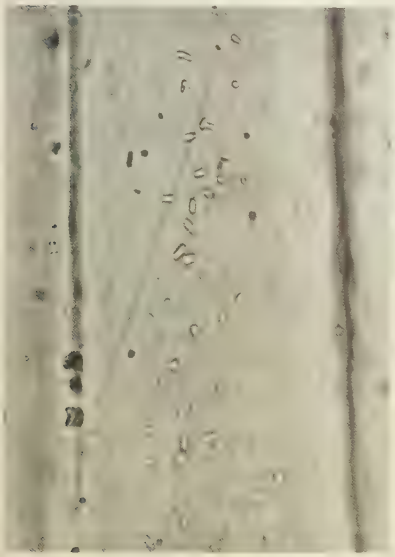
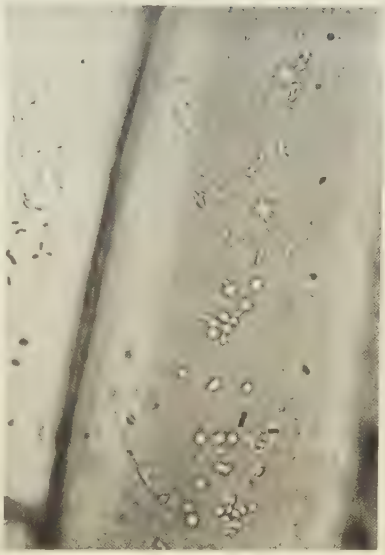


Fig. 1.



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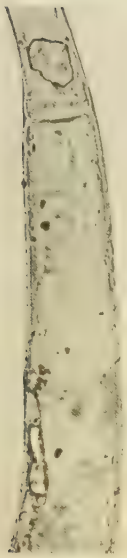


Fig. 3*a*.

Fig. 3*b*.

Fig. 4*a*.

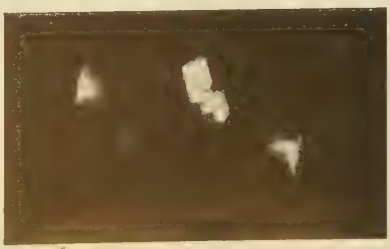


Fig. 4*b*.

BAWDEN AND SHEFFIELD.—THE INTRACELLULAR INCLUSIONS OF SOME PLANT VIRUS DISEASES (pp. 102–115)

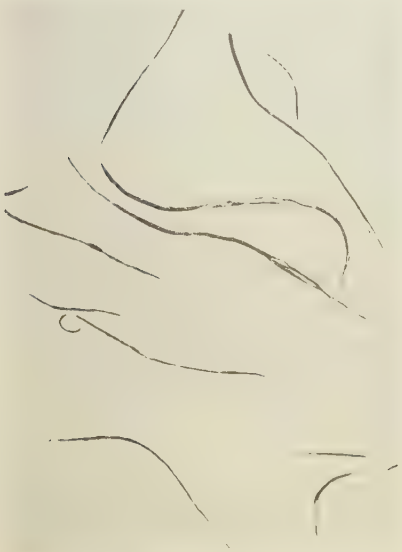
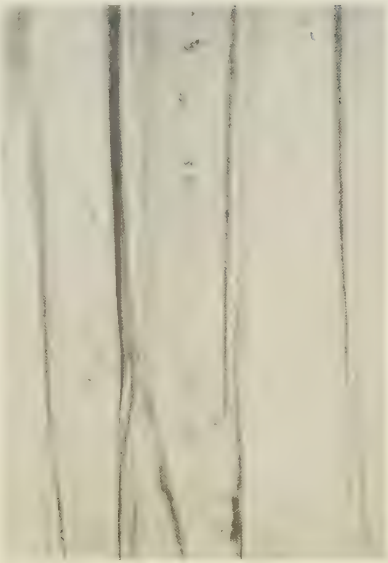


Fig. 1.



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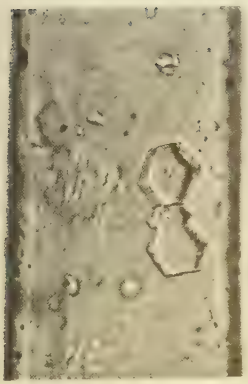

Fig. 2.

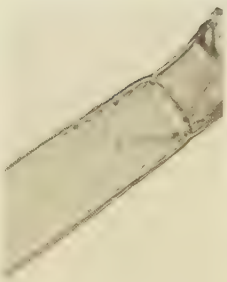

Fig. 4.

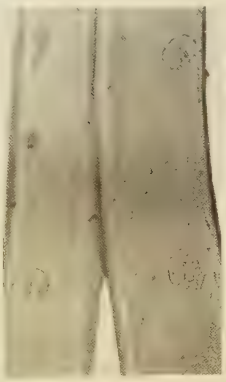

Fig. 5.

BAWDEN AND SHEFFIELD.—THE INTRACELLULAR INCLUSIONS OF SOME PLANT VIRUS DISEASES (pp. 102–115)

EXPLANATION OF PLATES VII AND VIII

Some of the conditions discussed have already been recorded photographically, and are figured in the papers of Beale, Clinch, Iwanowski and Sheffield cited.

The photographs were taken with a Leitz "Makam" camera. For Plate VII, figs. 1 and 2, a Leitz apochromatic 2 mm. objective (N.A. 1·4) was used in conjunction with a Leitz 10 × periplanatic ocular, giving a magnification of 900. For the other figures, a Leitz 6L achromatic objective (N.A. 0·65) was used together with a 10 × periplanatic ocular giving a magnification of 450. Contact prints are reproduced without alteration in size.

All preparations were unstained, and except for Plate VII, figs. 1 and 2, the cells were living when photographed.

PLATE VII

Figs. 1, 2. Healthy *Nicotiana tabacum*. Epidermal cells from beneath a vein. The vacuoles of both cells contain numerous particles many of which are rod-shaped and bear a superficial resemblance to bacteria. In addition fig. 2 also shows octahedral crystals, probably calcium oxalate. (These preparations were first examined in the living condition, but Brownian movement was too great for accurate photographs. Acetic alcohol was therefore run under the cover glass and the photographs taken immediately the cells were fixed. The process of fixation was closely watched but except for a slight shrinkage of the cytoplasm no artefacts were observed.)

Figs. 3*a*, *b*. Hair of *Lycopersicum esculentum* infected with Enation mosaic virus. In the upper cell an irregular crystalline inclusion is seen lying flat; in the lower cell a similar inclusion is seen edgeways. Fig. 3*a* taken with transmitted light and fig. 3*b* in polarized light. The cross cell wall and the inclusion lying edgeways are birefringent, but the inclusion lying flat is not.

Figs. 4*a*, *b*. Hair cells of *Solanum nodiflorum* containing crystalline material produced from disintegrating amorphous inclusions. Fig. 4*b* photographed between crossed Nicol prisms. The crystals in the upper cell are lying edgeways and are birefringent.

PLATE VIII

Fig. 1. Insoluble mesomorphic fibres, produced by the addition of a neutral solution of clupein sulphate to a purified preparation of Aucuba mosaic virus.

Fig. 2. Hair cell of *Nicotiana tabacum* infected with tobacco mosaic virus. Two plate-like crystals are seen, one being almost a perfect hexagon and the other slightly irregular.

Fig. 3. *N. tabacum* infected with tobacco ring-spot virus. Epidermal cells from beneath the vein containing numbers of small crystalline blocks.

Fig. 4. Hair cell of *N. tabacum* infected with Hyoscyamus virus 3. The amorphous inclusion is disintegrating and producing long needle-like fibres.

Fig. 5. *Lycopersicum esculentum* infected with the bushy stunt virus. Two hair cells are seen each containing a single amorphous inclusion body.

(*Received* 24 *August* 1938)

STUDIES ON APHIDES INFESTING THE POTATO CROP

VII. REPORT ON A SURVEY OF THE APHIS POPULATION OF POTATOES IN SELECTED DISTRICTS OF SCOTLAND (25 JULY–6 AUGUST 1936)

By the late W. MALDWYN DAVIES, B.Sc., Ph.D.[1]

University College of North Wales, Bangor

(With 1 text-figure)

CONTENTS

[1] Dr W. Maldwyn Davies died in February 1937, after preparing the first draft of this Report; and it has therefore fallen to me, as one very closely associated with him in his ecological work on aphides, to prepare the Report for publication. I have endeavoured simply to make the necessary verbal corrections which, I feel confident, Dr Davies himself would have desired. An abridged account of the investigations appeared in the *Scottish Journal of Agriculture*, July 1938.

T. Whitehead, University College of North Wales, Bangor

Introduction

The results of the survey of the aphis population on potato crops in selected districts in Scotland cannot be fully understood without a brief summary of the findings of the ecological studies on the aphis problem which led to these extended investigations.

Problem in north Wales. In north Wales there are two different types of district, one in the eastern portion where the spread of virus infection has been proved to be rapid– amounting to 33 % infection developing within two years in initially healthy stocks (Currie, 1933); the other, mainly in western districts, where, with roguing, there has been no increase in virus infection among the potato stocks since the establishment of the Welsh Seed Potato Scheme in 1928. It has been the purpose of the entomological studies to compare the aphis population in these two areas, in which it has been found there is a striking contrast (Davies, 1934). The standard method, which was devised for these comparative studies, is to count the number of aphides per 100 (lower) leaves, taken at random as the crop is traversed to and fro, as the index figure of the aphis population. By this method it was found that the index figure for the important insect vector *Myzus persicae* Sulz. always exceeded 100 and more commonly approached 1000 vectors/100 leaves in the district where the spread of viruses was rapid. On the other hand, in areas where little or no spread occurred, the number of *M. persicae*/100 leaves seldom exceeded 20, and often none was recorded. It was found that the optimum period for these surveys was mid-July, when the aphis population was usually approaching a maximum. Surveys in June and September were practically useless.

Experimental results. Having established this difference in the population in the two districts, it was necessary to ascertain what factors were responsible for the striking contrast occurring in districts not more than 100 miles apart.

The effect of temperature upon insect activity is well known and, since it was found that winged *M. persicae* fly readily at temperatures above 65° F., it was evident that such temperatures would be prevalent, in both districts, on most days in June and July when the aphides were migrating.

The influence of humidity upon aphis migration was unknown, but the mean monthly figure for relative humidity in the two districts contrasted markedly: the more humid districts of south Caernarvonshire, with lichen and moss-covered trees and sea mists, having a much higher figure than the drier districts of Flintshire (Davies, 1935*a*). Experiments

were, therefore, carried out to ascertain the effect of humidity upon the flight of these insects. In the drier atmospheres the aphides flew almost incessantly, but flight ceased in the higher humidities above 80% (Davies ,1935*a*).

Experiments were next undertaken to find out the effect of wind velocity upon migration (Davies, 1936), and it was surprising to discover that, whereas the aphides flew continuously when the air was still, the flight of *M. persicae* ceased completely as soon as the wind velocity reached only 4 m.p.h.

By means of a mechanical insect trap (Davies, 1935*b*), revolving and catching insects continuously near the meteorological station at the College Farm near Bangor, it has been possible to study the effect of combined meteorological factors upon migration. The results up to the present time indicate that aphis migration only attains appreciable intensity under the following combination of meteorological factors, viz. when the temperature exceeds 70° F. with a wind (or rather light breeze) velocity below 5 m.p.h., and when, in north Wales, these come from an easterly direction, from which point of the compass all breezes are dry.

Differences in meteorological conditions in the two areas, therefore, partly explained the contrast in aphis population on the respective potato crops, and there can be no doubt that the frequency of favourable weather conditions for flight during late May and June, when the aphides are flying to the potato crops, and again in September, when they are flying back to the winter hosts, has a very marked influence on the subsequent population of aphides on potatoes.

Importance of winter host plants. Meteorological differences did not prove to be the whole story, however, and a careful study of the life history of the aphis vector was necessary. It will be clear that with an annual crop such as potatoes, which is free from aphides when it appears above ground (Davies, 1932), the source of the aphides *before* they arrive on the potatoes will have an important bearing on the aphid population. *M. persicae*, like many other aphides, is known to over-winter in the egg stage on hardwood trees, in this case nectarines and peaches.

It was obvious from the distribution of the aphis in early summer that these plants could not account for all winged forms which arrive on potatoes in June. A search was made for other possible hardwood hosts but without success. Then a chance inspection of savoy cabbages at a provincial market in December showed them to be literally smothered with wingless *M. persicae*. Subsequent examination has proved that cruciferous plants, particularly winter Brassicae, harbour large numbers

of this species of aphis. In north Wales, at least, there can be no doubt that such plants as savoys, cabbages, brussels sprouts, swedes and turnips afford the main source of this insect vector in spring (Davies, 1934). Although the actual individuals arriving on Brassicae from potatoes in October die before spring there is a gradual reproduction of wingless generations on these crops throughout the winter (Davies & Whitehead, 1935). On the advent of warm weather, reproduction increases rapidly, and quite early a few winged forms may be produced which fly to seedling charlock and other weeds. Then the main migration of winged forms from the cruciferous plants takes place in late May and June. These migrating alatae readily find even single potato plants and, subsequently, produce several generations of wingless forms (Davies & Whitehead, 1938). Even the wingless aphides have been found to be in constant movement within the potato crop (Davies, 1932), and this constitutes a factor of considerable importance in the spread of virus diseases within any one crop. When the potatoes mature in August and September, winged forms are produced on the dying potato foliage and then complete the life cycle by a flight to the winter host plants.

The importance, therefore, of proximity of winter Brassicae in determining the initial aphis population on potatoes becomes obvious. There is, indeed, ample evidence to demonstrate that in market-garden districts, or in the vicinity of towns and villages where there are many gardens, there is invariably a considerable increase in the aphis population of potatoes as compared with districts remote from Brassicae crops. The eastern districts of north Wales include market-garden areas and border on the large market-garden districts of Cheshire and Lancashire. Further, the drier easterly breezes come from these latter areas and thus, no doubt, contribute to the high aphis population of potatoes in the eastern districts of north Wales as compared with that found in the western areas.

The present survey

Aphis populations at each centre. The purpose of the survey in Scotland was to ascertain whether the results of the investigations in north Wales could be applied to other districts. Scotland was selected because of the importance of the potato crop in that country, and in order to note any differences which might be attributable to the more northerly latitude. In the time available (25 July–4 August) it was not, of course, possible to do more than study the aphis population in a relatively few selected areas. It was, therefore, decided to restrict the observations to the eastern areas of Scotland where the comparison with north Wales might

yield the more interesting data in view of the easterly sea breeze being laden with moisture in contrast to the dry easterly winds of north Wales.

The 1936 season was a rather late one, and it was this fact which decided the slightly delayed period (25 July–4 August) selected for the survey. Wherever possible an early and a late variety of potato were examined at each centre and an effort was made to include the same

Fig. 1. Positions of centres in Scotland referred to in Table I.

varieties wherever a choice was available. This latter seemed advisable although it has been established (Whitehead *et al.* 1932) that there is no definite varietal selection by aphides, and that the time of appearance above ground, and the condition of the plants when migration takes place, are of greater importance than variety in determining the degree of aphis infestation.

The detailed results of the survey, with aphis species and population on each leaf examined at each centre, have been filed, and the accompanying table contains a summary of these observations. The positions of the centres in Scotland are shown in Fig. 1.

Table I. *Data collected on survey*

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number *M. persicae*/100 leaves	Other spp. 100 leaves	Remarks
			Midlothian 25 July 1936					
1	2 miles W. of Edinburgh	Open to W. & S. Easterly slope 200 ft.	Great Scot	5	3	5	—	—
2	Do.	200 ft. Open to E. Flat	Alness	19	6	19	—	—
3	Nursery E. of Edinburgh	264 ft. Many roses* growing during winter	Duke of York	361	90	100	258 *Mac. gei*	* Winter host of *M. gei*
4	2 miles E. of Edinburgh separated by slight hill	200 ft. In village. Flat	Great Scot	121	31	79	24 *Mac. gei* and 18 *M. pseudosolani*	
5	2 miles E. of Edinburgh	100 ft. Hill 823 ft. to W. near housing scheme	Epicure	125*	70	90	35 *Mac. gei*	* On basis of 50 leaves
6	5 miles S. of Edinburgh	850 ft. Upland plateau with high Pentland hills to N.W., hills 600–800 ft. to N., other hills E. and S.	Eclipse	8	2	2	6 *Mac. gei*	
			Arran Crest	1	1	1	0 *Mac. gei*	—
			East Lothian 28 July 1936					
7*a*	3½ miles S.W. of Dunbar	20 ft. Sea and marsh 1 mile to N.E., land rising to mountain 3 miles W.	Dunbar Cavalier	21	10	21	—	—
7*b*	Do.	Field more inland; swedes grown on adjacent field until late April–May when they had much foliage	Dunbar Yeoman	196*	50	186	—	* On basis of 60 leaves
8	5 miles S.E. of Dunbar	450 ft. Hills (900 ft.) N.W., short valley running E. to W. with mountains 1 mile to S.	Arran Banner	29	6	6	25 *A. rhamni*	—
			Fifeshire 26 and 27 July, 1936					
9*a*	2 miles S. of Kirkcaldy	200 ft. Field mainly open with hillock to side of crop. Sea 1 mile to E. and 2 miles S. Open land to W.	Doon Star	22	10	19	3 *A. rhamni*	* On basis of 50 leaves
			King Edward*	46	10	16	30 *A. rhamni*	
9*b*	Do.	Field near shore with easterly slope. Sea ¼ mile E. and S. Well exposed	British Queen	5	5	5	—	—
10	1 mile S. of Lady-bank and village of Kettle ¼ mile S.	150 ft. Undulating country to W. Sea 20 miles to E.	Sharpe's Express	67	28	28	31 *M. pseudo-solani*	—
			Great Scot	56	32	25	19 *M. pseudo-solani*	—

Table I (*cont.*)

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number *M. persicae*/100 leaves	Other spp./100 leaves	Remarks
			Fifeshire (*cont.*)					
11	2½ miles W. of Kinross	420 ft. Open plain	Catriona	1	1	0	0	—
			Arran Consul	1	1	1	0	—
12	Adjoining Auchtermuchty	300 ft. Many gardens to N.W.	Kerr's Pink	176	48	45	76 *Mac. gei*	—
13	2 miles W. of Milnathort	400–500 ft. Open district to E. and W.	Great Scot	1	1	0	1 *Mac. gei*	—
			May Queen	8	3	2	6 *Mac. gei*	—
14	4 miles N. of Cupar	100–200 ft. Open field in slight valley running E. to W. No villages near	Arran Pilot	10	2	2	1 *M. pseudosolani*	6 *Mac. gei*
			Majestic	6	2	2	4 *M. pseudosolani*	0 *Mac. gei*
			Duke of York	5	2	2	6	0
15*a*	4 miles N.E. of Newport	500–600 ft. On steep slope of N.E. facing Firth of Tay	Arran Pilot (500 ft.)	0	0	0	0	—
			Arran Pilot (600 ft.)	0	0	0	0	—
15*b*	Do.	Lowland fringe of Firth of Tay	Arran Pilot	6	2	6	0	—
			Ballydoon	4	2	4	—	—
			Perthshire 30 July 1936					
16	2 miles S. of Crieff	150 ft. Inland basin with high trees form ¾ mile circle around field. Mountains to W. and N.	Majestic	1	1	1	0	—
17	Adjoining Crieff	300 ft. Nursery S.E. of town with field sloping to S.E. Rose trees grown in quantity	Kerr's Pink	20	14	5	14 *Mac. gei*	—
18	2½ miles E. of Perth	200 ft. On ridge running between Perth and New Scone	Great Scot	29	11	18	0	—
19	Adjoining Perth	100 ft. Alongside mansion and other gardens. Field sloping S.W.	Duke of York	215	36	113	95 *Mac. gei*	—
			Angus 31 July 1936					
20*a*	1 mile E. of Dundee	154 ft. Open field with housing scheme encroaching	Majestic	191	51	77	96 *Mac. gei*	—
20*b*	Do.	60 ft. S.E. fringe of Dundee among houses. Sea less than ¼ mile to E.	Ballydoon	394	64	302	92 *Mac. gei*	—
21	Dunnichen	380 ft. Flat field with open land to W. Hills rising to 500 ft. to S., nearest village 1 mile to E.	Sharpe's Express	10	5	51	9 *Mac. gei*	—

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number *M. persicae*/100 leaves	Other spp./100 leaves	Remarks
			Angus (*cont.*)					
22	5 miles N.W. of Brechin	436 ft. Upland plain running slightly down from S.W. to N.E. Hills and mountains to N. and plain to S. No villages near	Arran Chief	0	0	0	0	—
			Great Scot	0	0	0	0	—
			Kerr's Pink*	1	1	1	0	* Kerr's Pink grown at 352 ft. in field bordered by trees
23	Farnell	82 ft. Inland plateau well wooded in parts but open to Montrose Basin to E. and with large deer park to N. and broken forests to W. No villages near	Arran Banner	0	0	0	0	—
			Arran Pilot	4	2	4	0	—
24	2 miles N. of Montrose	178 ft. Sheltered from town by slight hill. No villages near. Open plain with sea 3 miles to E. and S.E. Slight hills to N. Open 10–20 miles W. and S.W.	Ally	0	0	0	0	—
			Majestic	2	2	1	0	—
			Kincardineshire 31 July 1936					
25	1½ miles N. of Laurencekirk	229 ft. Open plain sweeping at foot of Grampians	Majestic	3	2	3	0	—
26	Fordoun	250 ft. At foot of Grampians which run W. to N. Hills rising to 860 ft. to S. and E. Open plain to S.W. No villages near	Majestic	2	2	1	0	—
			Duke of York	9	1	0	9 *A. rhamni*	—
			South Aberdeenshire 2 August 1936					
27	5 miles W. of Aberdeen	300 ft. In experimental plots. Hills rising to 800 ft. to N.W., W. and S.W. Open to N.	Arran Pilot	15	5	15	0	—
			Great Scot	21	7	18	0	—
28	3 miles N.W. of Aberdeen	200 ft. Hills 280 ft. to S.E., undulating hills rising to 250 ft. to E. with sea 3 miles beyond	Arran Banner	2	3	2	0	—
			Majestic	3	3	2	0	—
29	Eastern fringe of Aberdeen	20 ft. Sea ¼ mile to E.	Majestic	171	38	77	64 *Mac. gei* 38 *A. rhamni*	—
30	¼ mile S.W. of Aberdeen	100 ft. Slight hillock protecting from S.W. winds	British Queen	51	21	26	24 *Mac. gei*	—
			North Aberdeenshire 3 August 1936					
31	1 mile N.W. of Huntly	400 ft. Field in inland valley with forest and mountain to N. Hills rising to 700 ft. to W. and E. Field with S.E. slopes. Swedes grown in previous year	Golden Wonder	5	5	5	0	—
			Kerr's Pink	3	3	3	0	—

Table I (*cont.*)

Centre	Location	Aspect	Variety	Number aphides/100 leaves	% leaves infested	Number *M. persicae* 100 leaves	Other spp. 100 leaves	Remarks
			North Aberdeenshire (*cont.*)					
32	¼ mile S. of Huntly	420 ft. Market garden on S.W. slope at mouth of valley extending W., bounded by hills to N. and W. and slight hills to E.	Duke of York	42	13	36	0	—
33	4½ miles S.W. of Fraserburgh	166 ft. Field sloping E. Sea 5 miles to N.E. and E. Hills sloping to 300 ft. to S. and W.	Duke of York Great Scot	0 0	* *	0 0	0 0	* 1 *M. persicae* taken on 20 leaves in gardens adjoining
			Banffshire 2 and 3 August 1936					
34	4 miles S.W. of Banff	350 ft. Open fields with sea 3 miles to N.E. Hills rising to 500 ft. ½ mile to S.W. and S.	Duke of York Majestic Kerr's Pink	0* 0* 0*	0 0 0	0 0 0	0 0 0	* 1 *M. persicae* taken on small patch of kale in adjoining garden
35	Boyndie	60 ft. Small valley facing N.E. with hills rising to 180 ft. to W. and 250 ft. to N.W. Sea 1½ miles to N. Not typical of district	Duke of York	24	4	3	21 *A. rhamni*	—
			Morayshire 3 August 1936					
36	S.W. fringe of Elgin	37 ft. Adjoining numerous gardens. Kale in next field in 1935. Slight N.W. slope	Golden Wonder	901	76	829	68 *Mac. gei*	—
37	1½ miles S.W. of Elgin	50 ft. Sheltered by woods on all sides. No cruciferous crops grown near during 1935	King Edward	58	13	27	31 *Mac. gei*	—
			Ross-shire 4 August 1936					
38	1½ miles S.E. of Beauly	21 ft. On fringe of Beauly Firth. Hills rising to S.W., W. and N.	Golden Wonder Duke of York	6 6	5 6	4 4	0 0	— —
39	Invergordon	20 ft. On flat basin of Cromarty Firth ½ mile to S. and E. Hills rising to mountains 2 miles to N. Hills to W.	King Edward Majestic	2 7	2 4	2 7	0 0	— —
40	2 miles E. of Portmahomach	109 ft. On peninsula exposed to sea to E. and W., land tapering to sea to N.	King Edward	6	4	5	0	—
41	1 mile S.E. of Portmahomach	100 ft. Open and exposed field	Great Scot	8	7	6	0	—
42	2 miles N. of Portmahomach	78 ft. On peninsula with sea to N. and E. and W.	Arran Pilot	5	2	4	4	—
43	Portmahomach peninsula	100 ft. 1 mile from apex of peninsula. Exposed	May Queen	3	2	3	0	—

Distribution of the different species of aphides

(*a*) *Myzus persicae* Sulz. Specimens of this important insect vector of virus diseases were found at all the forty-three centres visited. In two cases (centres 33 and 34) none was actually included in the sample of 100 leaves (which, however, is not intended to give an absolute figure of population), but individuals were found in adjoining gardens. This species was taken almost at sea-level and also at the highest centre visited (850 ft.); it was common both in the north and the south. The special problem of the association of *M. persicae* with winter Brassicae is dealt with later. The index figure of population ranged from 0 individuals/200 leaves at centres 15*a*, 22 and 34, to over 100 individuals 100 leaves at centres 3, 7*b* and 19; with exceptionally high figures of 302 at centre 20*b*, and 829 at centre 36.

The development of the life history of this species in the east of Scotland seems to be similar to that found in north Wales. Only fifteen of the 2154 *M. persicae* collected were winged forms and, since wing buds were present on other specimens, it suggested that even these fifteen alatae had been produced on the potato crop on which they were collected. These facts point to a main migration of winged forms having taken place in June or earlier and, as is the case in north Wales, these winged parents subsequently died by the end of July. The heavier infestation, in general, of the earlier maturing varieties this year also indicated an early migration of the aphis.

(*b*) *Macrosiphum gei* Koch. Although this species has been recorded as a vector of leaf-roll in potatoes in America (Schultz & Folsom, 1925) and Holland (Elze, 1927), it has failed to transmit the virus in experiments under glass in this country (Smith, 1929, 1931). *M. gei*, which is often very common on potatoes in this country, over-winters in the egg stage on roses, and is later in arriving on potatoes than is *Myzus persicae*.

It was recorded at twenty-four of the centres; in low and high altitudes, and in the north as well as in the south. The index figures ranged from 1 to 258/100 leaves: the latter figure being obtained at centre 3 where rose trees were grown in large numbers in the nursery.

(*c*) *Aphis rhamni* Boyer. This species, like the last one, is recorded as a vector of leaf-roll and mosaic of potatoes in U.S.A. and Holland, but there is no evidence of transmission by *A. rhamni* in this country (Smith, 1931). The frequency and the numbers of this species found on potatoes during the last few years indicate a need for further transmission studies in this country. Little is known of its life cycle in this country except

that the buckthorn, which is its normal winter host, is rare in north Wales, and could not supply the numbers of the species appearing on potatoes. However, its habit of remaining in colonies on the same leaf, rather than moving to other foliage, would probably reduce its importance as a vector of potato viruses, as compared with *Myzus persicae*. During the present survey it was taken at ten centres, but nowhere did it exceed 30 individuals/100 leaves.

(*d*) *Myzus pseudosolani* Theob. This species is stated (Smith, 1929, 1931) to be a vector of potato leaf-roll, and possibly also of potato mosaic. It is seldom present in large numbers and little is known of its life history. It was recorded during the survey at twelve centres, and only in one instance did it attain 31/100 leaves.

Subsequent development of aphides at East Craigs and Ainville

It was, of course, impossible to keep continuous observation on the aphis population at the Scottish centres, so that the writer is the more indebted to Mr George Cockerham for undertaking this task as regards East Craigs and Ainville near Edinburgh. It is clear from his observations that the few aphides present at the time of the visit of the writer in July continued to reproduce until September. The counts made on plots at East Craigs showed that maximum infestation was reached about 10 September when the index figure was 800 *Myzus persicae*/100 leaves. At Ainville the maximum infestation of 500/100 leaves was reached on 5 September. This late development of wingless forms *within* the crop would, of course, only be of importance in spreading any virus infection already existing in the crop. It would be of great interest to ascertain whether this late development is an annual feature in certain districts in Scotland; though in 1936, at least, it is clear that at centres 20*b* and 36 the aphis development had begun early. In north Wales a late development is characteristic of the seed-producing districts.

Relationship of proximity of winter hosts to aphis population

The fact that *Myzus persicae* generally hibernates on winter Brassicae suggests the potential danger of growing potatoes, especially for seed, in the neighbourhood of such crops. Hence the importance of market-garden areas, villages, allotments, etc., which yield enormous numbers of winged forms in spring. This influence of proximity to gardens, etc., on the aphis population of potatoes was clearly demonstrated during the survey. Commencing in Midlothian, it will be seen from the tabulated

data that the heavy infestation at centre 3 occurred under nursery conditions where a quantity of winter hosts would be grown in close proximity. It will also be noted that this centre differed from all others in that *Macrosiphum gei* formed the predominant species: the winter host plant (roses) being grown in large numbers. The index figure for *Myzus persicae* (100/100 leaves) was also high owing to the proximity of winter Brassicae. In Fifeshire, the highest aphis populations were recorded at centres 10 and 12, which were in close proximity to villages. In Perthshire, the importance of this factor was again clear, for at centre 19, which bordered the east of Perth, 215 aphides were taken per 100 leaves as compared with 29 taken in a crop on a ridge 2 miles farther east. Even at Crieff, with its low infestation, a definite increase was found under market-garden conditions (compare centres 16 and 17).

In Angus the role of this factor was again evident, for at centres 20*a* and 20*b*, which are on the eastern outskirts of Dundee, index figures of 191 and 394 aphides/100 leaves were recorded, the higher figure being obtained practically in the town. In Aberdeenshire the story is continued, centres around Aberdeen being visited in order to test this factor. To the west of the city (centres 27 and 28) the aphis infestation was not very high (the index figures ranging from 3 to 21/100 leaves), but on the eastern border of the city (centre 29) 171 aphides were recorded per 100 leaves. The significance of the higher figure on the east at this and other centres will be referred to later. Even in an inland district like Huntly, the increase in aphides under market-garden conditions is seen in centre 32. In Morayshire, a further extreme case of the important influence of towns is seen at centre 36, which gave the highest figure found during the survey, for 901 aphides were taken on 100 leaves. The field at this centre extended almost to the town.

There can, therefore, be no doubt of the importance of market gardens and private gardens in determining the aphis population on neighbouring field crops of potatoes. This had already been found to be the case also in north Wales. As a basis of comparison it may be mentioned that in the market-garden areas near Holywell and Chester the total aphis population/100 leaves on 15 July 1936 amounted to 271 and 757 respectively. Again, confirmation was found at Ormskirk Seed Testing Station, in the centre of the market-garden area. Here, the phenomenal number of 1782 aphides/100 leaves (50 actually counted) was found on mixed varieties on 6 August 1936. Of these, 1092 were *M. persicae* and 690 *Macrosiphum gei*. Every lower leaf examined was infested and as many as 65 aphides were counted on a single leaf.

Another factor in connexion with proximity of winter host plants appeared to have greater importance in eastern Scotland than is the case in north Wales. Swedes and turnips in north Wales are not a potential danger as winter hosts because most of these crops are carted from the field and placed in pits, or fed off, before January. During the survey in Scotland, however, the higher infestation at centre 7*b*, with no village near, required further investigation, and was only explicable in the light of the practice in this district—as in others—of leaving swedes, turnips and kale in the field until spring. Sometimes the practice of "laying", or "Scheuching" as it is called locally, is carried out, i.e. the swedes are thrown into rows and lightly covered with soil. Whether this is done or the roots are left in the field there is considerable growth of foliage in early spring, even extending into May, on which *Myzus persicae* could hibernate. Observations made during the survey suggested that the presence of the aphis vector on potatoes could often be correlated with that of cruciferous crops during the winter months. In view of the practice of growing "special stock" seed alongside or in the same fields as swedes, it would be of value to ascertain what part these crops really do play, in Scotland, in providing the initial population of *M. persicae* on potatoes in spring.

Relationship of meteorological conditions to aphis population

The effect of meteorological conditions upon the migration of aphides in a given district requires prolonged investigation but, from the studies carried out in north Wales which were reviewed in the Introduction, it seemed likely that the frequency of *dry*, *light* breezes with temperatures above 65° F. would determine the extent of the migration to potatoes in late May and in June. The meteorological records in Scotland for June 1936, when the main migration of winged aphides would take place, were examined to see if any correlation between meteorological factors and aphis population was apparent. For this purpose, centres where there was an ample supply of winter hosts, such as can be found in market gardens and villages, were selected. From field observations referred to previously, it was clear that only days on which the maximum temperature exceeded 65° F. should be considered and, of these, days with wind velocities below 5 m.p.h. and relative humidities below 75 % would be the occasions on which migration of winged aphides would occur. Unfortunately, nowadays, the available meteorological data generally include only wind and humidity records taken at 9 hr. G.M.T., which is not the

time of day when general aphis migration takes place. There are, however, certain indications in the studies made during the survey which seem worthy of record.

Centres of high aphis infestation

(i) *Centre* 36, with 901 aphides/100 leaves. The figures from the meteorological station at Forres show that, of the 43 visited, this centre had the highest number of days (18) in June with maximum temperatures above 65° F. and on 13 of these days the wind velocity at 9 a.m. was below 5 m.p.h. Further, it was found that, even so early in the day, the relative humidity was below 75% on 13 occasions; this also being the highest number of such days found to have occurred at any centre visited.

(ii) *Centre* 20*b*, with 394 aphides/100 leaves. Data from Dundee showed that this centre had 17 days in June with maximum temperatures above 65° F., and on 11 of these the wind velocity at 9 a.m. was below 5 m.p.h., whilst 12 of the 17 days also showed a relative humidity below 75% at this hour.

(iii) *Centre* 3, with 361 aphides/100 leaves. This nursery on the east of Edinburgh was exceptional in that *Macrosiphum gei* predominated and, owing to the presence of rose stocks (its winter host) in the same nursery, it suggested a very local migration. Probably centres 4 and 5 (with 121 and 125 aphides/100 leaves respectively) are more representative of fields within urban areas. The meteorological data from Edinburgh, approximately 2 miles to the west of these three centres, showed that on 10 days in June the maximum temperature exceeded 65° F., and of these 9 had wind velocities below 5 m.p.h. at 9 a.m., and 6 days had a relative humidity of less than 75%.

(iv) *Centre* 19, with 215 aphides/100 leaves. Data from Perth showed that on 16 days in June the maximum temperatures exceeded 65° F., and on 10 of these the relative humidity was below 75% at 9 a.m. There were, however, only 5 days when the wind velocity was below 5 m.p.h., and therefore suitable for aphis flight. On the other hand, this centre adjoined a number of gardens, and was itself sheltered, so that conditions were optimum for the rapid breeding up of the progeny of such aphides as reached the potatoes as migrants (compare centre 18 below).

(v) *Centres* 10 *and* 12, with 67 and 176 aphides/100 leaves respectively. These two centres were near to villages some 9 miles from Cupar, which town provided meteorological data showing that 16 days in June had a maximum temperature above 65° F. and had a wind velocity at 9 a.m. of less than 5 m.p.h., whilst on 11 of these days the relative humidity at

9 a.m. was below 75 %. It is unfortunate that no count was taken under market-garden conditions in the immediate vicinity of Cupar.

Centres of low aphis infestation

(i) *Centres* 22–26, with aphis counts not exceeding 9/100 leaves. In the Montrose district, although market gardens were not inspected, the gardens near most of the farms were examined and supported the comparatively low aphis counts made at centres 22, 23, 24, 25 and 26. Meteorological data from Montrose showed that only on 6 days in June did the maximum temperature exceed 65° F. and there were only three occasions when the relative humidity at 9 a.m. fell below 75 %, whilst no single day had a wind velocity at 9 a.m. below 5 m.p.h.

(ii) *Centres* 33 *and* 34, with 0 aphides/100 leaves. The meteorological data from Banff indicated that, whereas the maximum temperature exceeded 65° F. on 13 days in June, only on 3 of these was the wind velocity at 9 a.m. below 5 m.p.h., and there were only 7 days when the relative humidity was below 75 %.

(iii) *Centres* 39–43, with not more than 8 aphides/100 leaves. The nearest source of meteorological data was at Fortrose, and clearly showed the high humidities prevailing in this area. Not a single day in June had a relative humidity at 9 a.m. below 75 % although the temperatures were favourable for flight on 13 days. Nine days in the vicinity of Fortrose had wind velocities below 5 m.p.h. at 9 a.m., but it is highly probable that this number would be less on the exposed peninsular conditions of Portmahomach where centres 40–43 were located.

(iv) *Centre* 18, with 29 aphides/100 leaves, deserves separate mention in that it was located within 2 miles of centre 19 (cf. (iv) above) where the conditions as regards temperature and humidity favoured flight, but a sufficiently low wind velocity occurred only on 5 days. This latter fact may be regarded as sufficient to explain the low count at centre 18 in contrast to the high count taken at centre 19.

Conclusions

It is desirable that studies on the effect of meteorological factors upon migration of aphides under Scottish conditions should be continued and amplified. Nevertheless, the results obtained during the present survey when viewed in the light of field observations at the College Farm, Bangor, in north Wales, lend considerable support to the conclusion that migration of aphides does not attain appreciable intensity unless the

temperature exceeds 65° F., the wind velocities remain below 5 m.p.h., and the relative humidities do not exceed 75 %.

The consistent increase in numbers of aphides in fields to the *east* of urban areas, as compared with that found to the *west*, strongly suggests that the winged forms migrated during periods of drier, southerly and west breezes from the land. In this case, therefore, the main migration would be in the opposite direction from that obtaining in north Wales, and would indicate that migration follows the direction of the drier breezes whether these blow from the west, as in eastern Scotland, or from the east as is the case in north Wales.

Discussion

Aphis population

It should be remembered that the two years, 1933 and 1934, previous to the present survey were "peak" years for aphis infestation, at least in north Wales, and this fact must necessarily limit the extent to which the results of the survey can be used as a basis for generalizing. Yet, the important fact remains that, even by taking random samples of 100 lower leaves, no centre was found where the potato crops were absolutely free from aphides, including *Myzus persicae* the vector of so many virus diseases. After some years' experience of species of aphides attacking potatoes, the writer doubts whether there is any locality in Great Britain in which potatoes are grown on any scale where aphides could not be found during the month of July. This opinion admittedly requires much further work before it can be fully substantiated, and it would be of real interest in this connexion if potato crops could be examined in such isolated areas as the Outer Hebrides during the month of July.

The role of the winter host plants in the development of the aphis population has been shown by this survey to be of importance in eastern Scotland, as has already been proved to be the case in north Wales. There is also an indication that, in Scotland, centres to the east of towns may be more heavily infested than those on the western side. This, it is suggested, is to be correlated with the fact (ascertained from meteorological records) that the light, drier breezes during June came, overland, from the west.

The survey from the seed-potato production aspect

That there are districts where aphis population on potatoes is comparatively small is as apparent from these studies in eastern Scotland as it is in north Wales. Further, there is ample evidence, from the viewpoint

of the dissemination of virus diseases, that "stock" seed of excellent health has been grown for years in such districts of low aphis infestation. Thus, in the light of these facts, it becomes unnecessary to search for large areas which are entirely free from aphides for the production of such special nursery stocks of exceptionally healthy seed potatoes. Greater advantage, however, might be taken of districts with low aphis infestation, with their comparative freedom from the potential danger of insect vectors, in producing special stocks which could be used to replenish other seed-producing centres, since these may, for economic or other reasons, have to extend into districts with a moderately heavy aphis infestation. On a small scale such a precaution is taken by this College under the scheme for producing seed potatoes in north Wales and, even in Scotland, it would be of value in ensuring that, during seasons of abnormal aphis infestation such as 1933 and 1934, there would be sufficient acreage of nursery stocks for later distribution. In this connexion it may be stressed that low aphis infestation is not of necessity associated with high altitudes, and an extension of seed-potato growing in selected low-lying wind-swept districts with high humidities is worthy of consideration. Further, it may be noted that districts even with low rainfall but with frequent sea mists will provide the degree of humidity unfavourable for aphis migration. In north Wales most of the successful seed-producing centres are low-lying and coastal.

The fact of the existence of such heavy aphis infestations as 901/100 leaves as far north as Morayshire stresses the need for insistence upon greater isolation of stocks grown for seed, even T.S. (H) stocks, than is afforded by a two-drill width of separation from potatoes with appreciable virus infection. Clearly, with such a population of aphides there is serious risk, since it has recently been proved that aphides find, and carry disease to, even single isolated potato plants from distances of at least a quarter of a mile (Davies & Whitehead, 1938). The use of swedes as the separating crop between potatoes in Scotland is also obviously open to criticism since this winter host supplies the means of over-wintering for the vector *Myzus persicae*. Indeed, the survey provides ample evidence to indicate the need for special precautions when seed-potato crops are grown in the immediate vicinity of market gardens and towns since, in addition to the probable presence in these districts of potato stocks of uncertain health, there is now the further evidence of higher aphis populations in such areas.

Finally, the survey has provided evidence of the considerable scope for entomological studies on insect vectors under Scottish conditions, and

it is hoped that the results of this survey will add a stimulus for some such investigation in the near future.

SUMMARY

1. This communication is the substance of a report on a survey of the aphis population in certain districts of Scotland carried out from 24 July to 4 August 1936.

2. Forty-three centres in eastern Scotland, from Midlothian in the south to Ross-shire in the north, were examined by the standard method for determining the aphis "index" figure.

3. The varying figures obtained have been analysed with a view to establishing any relation there might be with (*a*) proximity to winter host plants, and (*b*) meteorological conditions such as temperature, humidity and wind velocity.

4. The results have been summarized in a discussion in which are indicated the special precautions necessary to ensure health in seed-potato stocks.

The survey was carried out with the aid of a special research grant from the Agricultural Research Council. Work of this character would be impossible without the sympathetic interest and support of the growers whose potato stocks were examined, as well as of all those responsible for agricultural advisory and research work in the areas selected. The writer particularly wishes to express his indebtedness to Mr T. Anderson, Director of the Seed Testing and Plant Registration Station, Corstorphine; Mr W. Robb, Director of the Scottish Society's Station for Research in Plant Breeding; and Dr Guy Morison, the Advisory Entomologist of Aberdeen University. He is equally conscious of his indebtedness to Mr George Cockerham for his notes on the subsequent development of the aphis population at two centres, and to Mr R. J. Scott of East Craigs as well as to Dr T. McIntosh and Col. A. S. Fortune, senior Inspectors of the Scottish Board of Agriculture, for help received by discussions.

REFERENCES

CURRIE, J. F. (1933). The production of high-grade seed potatoes in north Wales. *J. Minist. Agric.* **40**, 316.

DAVIES, W. M. (1932). Ecological studies on aphides infesting the potato crop. *Bull. ent. Res.* **23**, 525.

—— (1934). Studies on aphides infesting the potato crop. II. Aphis survey: its bearing upon the selection of districts for seed-potato production. *Ann. appl. Biol.* **21**, 283.

Davies, W. M. (1935*a*). Studies on aphides infesting the potato crop. III. Effect of variation in relative humidity on the flight of *Myzus persicae* Sulz. *Ann. appl. Biol.* **22**, 106.

—— (1935*b*). A water-power mechanical insect trap. *Bull. ent. Res.* **26**, 553.

—— (1936). Studies on aphides infesting the potato crop. V. Laboratory experiments on the effect of wind velocity on the flight of *Myzus persicae* Sulz. *Ann. appl. Biol.* **23**, 401.

Davies, W. M. & Whitehead, T. (1935). Studies on aphides infesting the potato crop. IV. Notes on the migration and condition of alate *Myzus persicae* Sulz. *Ann. appl. Biol.* **22**, 549.

Davies (the late), W. M. & Whitehead, T. (1938). Studies on aphides infesting the potato crop. VI. Aphis infestation of isolated plants. *Ann. appl. Biol.* **25**, 122.

Elze, D. L. (1927). De Verspreding van Virusziekten van de Aardappel door Insekten. *Meded. LandbHoogesch., Wageningen.*

Schultz, E. S. & Folsom, D. (1925). Infection and dissemination experiments with degeneration diseases of potatoes; observations in 1923. *J. agric. Res.* **30**, 493.

Smith, K. M. (1929). Studies on plant virus diseases. V. Insect transmission of potato leaf-roll. *Ann. appl. Biol.* **16**, 209.

—— (1931). Studies on plant virus diseases. IX. Some further experiments on the insect transmission of potato leaf-roll. *Ann. appl. Biol.* **18**, 141.

Whitehead, T., Currie, J. F. & Davies, W. M. (1932). Virus diseases in relation to commercial seed-potato production. *Ann. appl. Biol.* **19**, 529.

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CRYPTORRHYNCHUS LAPATHI L. IN RELATION TO THE WATERMARK DISEASE OF THE CRICKET-BAT WILLOW

By EDWARD McC. CALLAN

Imperial College of Tropical Agriculture, Trinidad, B.W.I.

The poplar and willow borer or weevil, *Cryptorrhynchus lapathi* L., is well known as a pest of willows in Europe, and sometime prior to 1887 was introduced into the United States, where it has become established. The weevil has been described by numerous authors, and is discussed in most works on forest entomology including those of Gillanders (1912), Doane *et al.* (1936) and Chrystal (1937). The most complete account of its biology is given by Matheson (1917). The weevil is probably widely distributed in Great Britain although somewhat local, and its range of host plants is fairly wide, including various species of alder, willow, poplar and birch.

The eggs are laid in the bark of branches of two or more years of age in cavities excavated by the proboscis. The young larva at first feeds exclusively in the bark and the cambium. Later, when nearly full grown, the larva leaves the cambium and burrows into the wood. In the smaller branches the larval channel often lies in the central pith region. Frass consisting of small particles of wood is forced from the larval chamber, and is very conspicuous on infested branches. The pupal chamber is formed at the upper end of the larval burrow, which may be several inches in length and is packed with frass. The fully fed larva pupates in this chamber, and the adult on emergence cuts its way through the frass to the exterior.

The length of the life cycle apparently varies greatly. There may be a complete generation every year, or the total life cycle may extend over a period of 2 years. Adults emerging in the late summer probably do not leave the branch, but hibernate within the pupal chamber until the following spring. On leaving the pupal cells in the spring the weevils begin to feed on the young green shoots. They are voracious feeders, a single weevil kept under observation making as many as sixteen feeding punctures in a shoot in 24 hr. There seems to be a distinct preference for the 1-year-old shoots, although feeding also occurs on woody shoots of greater age. Attacked shoots usually bend over in a characteristic way or

are broken by the wind. When disturbed the weevils feign death and drop to the ground, and on being handled emit a squeaking sound.

The weevil is usually responsible for some damage in most areas where osiers are grown, but few records appear to exist of the infestation of the cricket-bat willow, *Salix alba* var. *caerulea* Smith. Dr H. F. Barnes informed me of the occurrence of the weevil in considerable numbers at Batford, near Harpenden, Herts, on *S. viminalis* L., and, although bat willows were situated nearby, they did not appear to be infested. Severe infestations of bat willows were observed in Kent in 1928 and 1929, and a piece of infested bat willow was received from Lyonshall, Hereford, in 1937. Haines (1937) records that at Linwood, near Ringwood, Hants, the weevil is extending its attacks from *S. viminalis* to the bat willow. On visiting this locality, the infestations were found to be severe, the larvae tunnelling in the main stems of many young trees. *S. caprea* L. was also found to be infested, but poplars and alders were unattacked.

S. alba var. *caerulea* yields the timber used in the manufacture of cricket bats. Trees are grown on an extensive scale in many parts of East Anglia, especially Essex and Hertfordshire, although in recent years the tree has been planted elsewhere. The bat willow is subject to the disease known as "watermark", caused by *Bacterium salicis* (Day), emend. Dowson (1937), which renders the wood useless for bat manufacture. *B. salicis* is apparently restricted to the wood of the tree, and insects, which feed upon or inhabit the wood during a part of their life cycle, are suspected of being involved in the transmission of the disease. In the case of *Cryptorrhynchus lapathi* it is the feeding habits of the adult weevil which are of chief significance as regards disease transmission, the species apparently being well adapted for the mechanical introduction of *Bacterium salicis* into the wood.

A very similar watermark disease of willows other than *Salix alba* var. *caerulea* was investigated in Holland by Lindeijer (1932). She ascribed the disease to *Pseudomonas saliciperda* Lindeijer, and stated that this disease must be considered identical with the watermark disease of the bat willow in England. She also showed that in Holland *Cryptorrhynchus lapathi* is closely associated with and is capable of transmitting the disease. Two infection experiments were made. In one of these three weevils were allowed to walk for several minutes on a pure culture of *Pseudomonas saliciperda* and then transferred to healthy branches of *Salix alba*. No infection resulted in this case. In the second experiment three weevils were allowed to feed on diseased wood and were then transferred to a healthy branch. Infection occurred in this experiment,

although *Pseudomonas saliciperda* was not isolated from the diseased wood. From this single positive experiment Lindeijer concluded that the weevil is capable of transmitting the disease.

An attempt was made to repeat Lindeijer's experiments in connexion with the watermark disease of the bat willow. In 1936 nine weevils were used in three types of experiments. Weevils were allowed to feed on diseased shoots, or their probosces were smeared with the bacterial exudate from a watermarked shoot or with a culture of *Bacterium salicis*. They were then transferred to the branches of two healthy bat willows and to a number of healthy pot plants, on which they fed voraciously. No symptoms of the disease were observable in these plants and trees during 1937 and 1938.

These infection experiments were repeated in 1937 using forty-six weevils altogether, the majority being allowed to feed on diseased shoots on watermarked trees for a number of days. They were then allowed to feed on healthy pot plants and on the branches of four healthy bat willows. No symptoms of disease have appeared in 1938.

Conclusion

Although *Cryptorrhynchus lapathi* has been found occasionally attacking the bat willow, it has not yet been associated with the watermark disease in the field. Infection experiments designed to show if the insect is capable of transmitting the disease have as yet given no positive results.

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REFERENCES

Chrystal, R. N. (1937). *Insects of the British Woodlands.* London and New York.

Doane, R. W., van Dyke, E. C., Chamberlain, W. J. & Burke, H. E. (1936). *Forest Insects.* London.

Dowson, W. J. (1937). *Bacterium salicis* Day, the cause of the watermark disease of the cricket-bat willow. *Ann. appl. Biol.* **24**, 5–28.

Gillanders, A. T. (1912). *Forest Entomology.* Edinburgh and London.

Haines, F. H. (1937). *Cryptorrhynchus lapathi* L. on *Salix alba* var. *caerulea* Sm. *J. Soc. Brit. Ent.* **1**, 194.

Lindeijer, E. J. (1932). *De Backterie-Ziekte van den Wilg Veroorzaakt door* Pseudomonas saliciperda *n.sp.* Baarn: Hollandia-Drukkerij.

Matheson, R. (1917). The poplar and willow borer. *Bull. Cornell agric. Exp. Sta.* no. 388.

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ENCHYTRAEID WORMS AND THE BACTERIA BED METHOD OF SEWAGE TREATMENT

By T. B. REYNOLDSON, Ph.D.
The University of Leeds

(With Plate IX and 4 Text-figures)

CONTENTS

PART I. FIELD OBSERVATIONS

Introduction

It has been realized from the earliest days of sewage bacteria beds that the larger organisms present, such as worms and insect larvae, play a significant part in the economy of the bed by scouring the medium and preventing the bed from becoming choked (Buswell, 1928). Few investigations have been made, however, to assess the parts played by the various organisms in the fauna.

Parkinson & Bell (1919) showed the capacity of the Collembolan, *Achorutes viaticus* Tulb., to keep open experimental bacteria beds, and advocated the deliberate employment of this insect. Welch (1914) made

observations on the behaviour of the Enchytraeid worm, *Lumbricillus rutilus* Welch, but did not consider its scouring action. Much work has been done at the New Jersey Agricultural Experiment Stations to determine the meaning of the natural sloughing of the zoogloeal growth which covers the medium. The beds concerned have a thin surface growth of *Stigeoclonium* (Isokontae) and *Oscillatoria* (Cyanophyceae) which shows no quantitative fluctuations. The dominant macro-organisms are nematodes, though Oligochaetes (Pristina and Aeolosoma) are also present. These organisms are said to increase prior to "off-loading" and to decrease afterwards. It is considered that their wriggling movements might loosen the film causing the sloughing (Rudolfs, 1924).

More recently Lloyd (1935, 1937) has made observations of the bacteria beds in the Leeds neighbourhood, where the surface growth is ordinarily a highly resistant layer of *Phormidium* (Cyanophyceae) with the capacity to become continuous when scouring organisms do not hold it in check. He has suggested that apart from the physical forces, such as sun and wind which rupture and tear off fragments of the film, the most important controlling factor may be the abundant Enchytraeid worms. The investigations here described were undertaken on the beds at Knostrop, Leeds, to test this suggestion, and to study more completely the conditions in the bed affecting the relations of the flora and fauna, and so afford a better understanding of the changes which are known to take place.

For this purpose the behaviour and distribution of the worms have been studied by field and experimental methods, involving the use of model beds.

The bacteria beds, their flora and fauna

A description of the bacteria beds

Bacteria beds vary in construction, the conditions of working, and the character of the sewage treated. Each of these factors may have an important bearing on the nature of the flora and fauna.

The Leeds beds are rectangular, 6 ft. deep and sunk below the surface. They contain pebbles composing the medium which are 2–3 in. across in the surface layer, becoming smaller in the lower layers, and still deeper, mixed with gravel. The liquid sewage is conveyed from the settling tanks to the bed and delivered as a sheet of water from travelling distributors of the Mill's type. Each machine supplies an area of bed 75 yd. long and 20 yd. wide, and it takes 10–15 min. to traverse this area. The liquid,

trickling slowly through the pebbles, drains into the bed effluent channels which carry it to the final settling tanks. When the sewage flow is of average volume the beds are worked 16–20 hr. per day, but longer or shorter resting periods, depending on the volume of sewage to be treated, may be given usually at night. Sometimes the beds may be rested for a few days, and then only the topmost few inches of the medium dry out.

Conditions in the bacteria bed

The beds form a peculiar and in some respects unique environment which has been described by Lloyd (1935). The outstanding features are the much reduced fluctuations in the temperature of the beds compared with those of the atmosphere, the constant saturation of the bed with moisture, and the great depth of the habitable zone compared with natural environments.

The temperature of the beds deserves special mention. It is relatively uniform from 6 in. downwards, probably due to the heat of vital activities taking place in the bed (Lloyd, 1935). 3–4 in. below the surface the temperature is much higher (2–4° C.) than that of the atmosphere in winter and correspondingly lower in summer. Frost is exceedingly rare below the surface of the bed.

The flora of the bacteria bed

The surface stones are covered by a growth of *Phormidium* (Cyanophyceae) often 2–3 mm. in thickness. It has pronounced seasonal fluctuations in character and quantity. In spring it becomes detached and washed away almost completely from the surface down into the bed. The stones beneath are covered by a slimy, jelly-like, zoogloeal growth of fungi and bacteria which contains numerous other micro-organisms, mainly Protozoa. This covering is also cast off in spring at the same time as the surface growth, the two processes being collectively termed the "off-loading" of the beds.

The fauna of the bacteria beds

Apart from the micro-organisms present, the fauna includes a number of Oligochaetes, and of these the Enchytraeid, *Lumbricillus lineatus* Mull., is the most important, but the Lumbricid, *Lumbricus rubellus* Hoff., is also abundant. The latter is confined to the deeper layers of the bed, and rarely found in the upper 6 in. However, the small immature worms of this species are frequently seen in the surface layers. The adults are most in evidence in the final settling tanks, reaching there

from the beds via the effluent channels. In spring they occur in such numbers as to cover the bottom of these tanks to a depth of several inches, where they putrefy, causing an unpleasant stench. Another Oligochaete belonging to the Naididae is also found in the effluent channels. This worm, which is essentially aquatic in habit, has not been found in the bed, but it may occur among the pebbles at the bottom of the bed.

The larvae of various insects form one of the most important groups, rivalling the Oligochaetes in abundance. The commonest are *Psychoda severini* Tonn. and *P. alternata* Say., *Metriocnemus longitarsus* Goet. and *M. hirticollis* Staeg., and *Spaniotoma minima* Mg.

Slugs are typical denizens, being rather numerous at Leeds, and a small snail, *Limnea glabra* Mull., is also found. Mites and spiders are common, the former occurring on dry patches of the pebbles. Nematodes may also be abundant in the surface growth and on the stones. All these organisms, with the exception of the Arachnids, are scourers of the medium, but relations between them may alter when food becomes scarce, and Lloyd (1937) suggests that this factor may be of importance in determining the variations which occur in the biological balance of the bed flora and fauna.

Description and habits of *Lumbricillus lineatus* Mull.

Description

Probably the most important organism contributing to the changes occurring in the bed is the worm *Lumbricillus lineatus*. It is pink in colour due to the haemoglobin in the blood, of small size, averaging 15 mm. in length and 1·25 mm. in diameter. It has approximately fifty segments. The general organization of the worm is much simpler than that of the Lumbricids. Apart from its size the chief distinction from typical earthworms lies in the number of setae per bundle which in this species is generally four to seven as compared with the two present in earthworms. The setae are ∫ shaped, arranged fan-wise, and occur in four bundles to each segment, two lateral and two ventral.

Examination of the diagnostic features shows that they agree most closely with those given for *L. lineatus* Mull., and this title is retained. Considerable doubt exists in the determination of the species of *Lumbricillus*; according to Beddard (1895) the genus contains nine species, but Michaelsen (1900) describes fourteen. Welch, studying the Enchytraeidae in America, decided that of the European species, *Lumbricillus lineatus* Mull., *subterraneus* Vejd., *litoreus* Hesse, *verrucosus* Clap., and *agilis*

(author?) were one and the same. Stephenson (1922), after discussing the variability in this genus and the need for care in dealing with it, comes to a similar conclusion.

Habits

The worms occur in fluctuating numbers in the surface growth, and exhibit marked seasonal migrations. They remain burrowed in the growth by day, but observations made throughout the night in August (1937) showed that during darkness they come on to the upper surface. They move actively away as the light increases and are not found exposed during the day, being strongly photofugic. Immediately below the topmost layer of pebbles the worms are found in large numbers roughly averaging 100,000-150,000 cu. ft., and persist in reduced numbers to the bottom of the bed where they are periodically washed out by the sewage. Their occurrence below the surface in discrete clumps of from twenty to 200 individuals is a characteristic feature. The centre or "nucleus" of each cluster consists of a piece of alga from the surface or of detritus. When the worms are cleaned of all foreign matter the tendency to cluster is much reduced. This pronounced thigmotactic response to alga and sludge has been remarked upon by Welch (1914) who carried out experiments demonstrating the fact. The clustering habit might be considered as a protection against desiccation, and Welch pointed out that resistance to desiccation was increased when the worms occurred in masses. But it has been observed on a number of occasions that when the beds become dry the worms, instead of remaining in clumps, generally separate showing that it is not adaptive in this respect. The larvae of the common flies inhabiting the beds are often found with the clusters of worms, but it is still uncertain whether they feed on the worms or on the particles.

The worms are not immersed in the sewage but only covered by a surface film, and there seems to be little doubt that they must use atmospheric oxygen for respiratory purposes in a similar manner to ordinary earthworms.

The cocoons of the worms are oval and measure approximately 1·1 by 0·75 mm. The walls are transparent and the white eggs can be seen inside distinctly. They are found embedded in the alga and also occur on the pebbles often very abundantly, one for instance, $2 \times 2 \times 1$ in., had forty-three cocoons attached. They are deposited in considerably larger numbers on pebbles with a rough surface, and groups of cocoons are found in the crevices. As already mentioned they are attached to the

substratum and can resist the pressure applied by a small paint brush to dislodge them. This fact is of importance since attachment prevents the cocoons from being washed out of the bed by the stream of liquid percolating through, and so enables the worm to maintain its numbers. It may be the limiting factor that keeps out other Enchytraeids, since the genus *Lumbricillus* is the only one of this family of Oligochaetes in which the cocoons are definitely stated to be attached to the substratum (Stephenson, 1930), but full details of the other genera are lacking. This factor is also of importance to the relative abundance of the different flies as pointed out by Lloyd (1937), and Graham (unpublished) working on these beds has shown that this is so.

Field and laboratory methods

The area selected for detailed study

Observations during the spring off-loading (January–July 1937) carried out on two beds showed that the changes were similar, so attention was concentrated on one for the remaining 9 months (August–May 1937–8). It was early apparent that the wind break afforded by a channelled wall, 4½ ft. high, running in a north to south direction, and carrying the sewage, was making an appreciable difference to the temperature of part of the bed during the colder months. The following records for two beds indicate the extent of this influence:

Bed	Close to wall	2 ft. on east side	4 ft. on east side	Middle of bed
2	7·6° C.	8·1° C.	8·0° C.	7·0° C.
3	7·8° C.	8·0° C.	8·3° C.	7·0° C.

The prevailing wind is westerly, so that such temperature differences commonly prevail. Since a difference in the growth of the alga had been noted in the sheltered region the quantitative estimations have been carried out in duplicate for 1 year, for what are now termed the "sheltered" and "exposed" parts of the bed, the former being an area extending for 6 ft. on the east side of the wall, the latter, the remaining area of the bed.

Field observations and methods for quantitative estimations

In view of the presumed importance of the worms in the bed, any investigation of the changes occurring at the surface and in the depths of the bed must include estimations of the abundance of the worms in the surface growth and also their distribution and movements in the bed. Therefore, routine observations have been made twice weekly, including a general survey of the condition of the alga, and particularly the abundance and distribution of the worms in it. An approximate estimate of the number of worms in the upper 6 in. of medium was made, and for convenience classified as follows: very abundant, abundant, common, and scarce. An examination of the pebbles for cocoons and insect larvae was also carried out.

The weather at the time of observation was recorded, and during winter and summer extremes a sample series of bed temperatures was taken at shallow depths. Daily temperature records of the atmosphere, bed surface, and at a depth of 30 in.

together with a record of the solids in the bed effluent, have been supplied by the Manager of the Leeds Sewage Works.

To determine the quantity of worms in the surface growth, 20 g. of it were collected at random over the bed into a jar. For estimating the number of worms in the bed below the surface, depths of 12 and 30 in. were taken. A satisfactory method for these depths included the use of lidded iron pipes 4 in. in diameter, sunk into the bed to the respective levels. Simple, open, muslin bags containing 20 g. of scalded alga were let down the pipe and rested on the medium. These bags were changed every week. The worms moved readily into them and fed upon the alga.

Quantitative estimations of the alga were obtained by scraping 1 sq. ft. of medium once a week. A wooden frame enclosing this area was used for guidance. The condition of this alga was also noted.

The number of worms washed out of the bed in the effluent was estimated from material supplied by Mr J. F. Graham, who carried out weekly strainings of the effluent for 5 min. periods over 1 year.

Treatment of the alga collected for quantitative estimations

It was necessary to separate the worms from the collected alga for counting, and some difficulty was experienced in forcing them out. Immersion in warm water or deoxygenated water proved inadequate, but similar treatment with a 0·05% solution of copper sulphate caused the worms to emerge before they were killed. The efficiency of this method was tested by examining treated alga, and less than 1% of the total number of worms remained concealed. In practice 20 g. of the alga were immersed in this weak solution and left overnight. Then the worms were teased free of it and counted under water in shallow glass dishes $10 \times 7 \times 2$ in., standing on black cardboard, ruled by white lines into 2 in. squares. The contents of the muslin bags were similarly treated. If the catch was reckoned to be less than 1000 the entire number was counted, if greater the worms were spread evenly over the bottom of the dish and one-quarter of the area counted. The material collected by straining the bed effluent was examined fresh in the shallow dishes.

The surface growth scraped from 1 sq. ft. of medium was transferred to filter paper, dried in an oven and weighed.

The reliability of the methods

Some idea of the accuracy of the methods employed for sampling was essential. The number of worms in the surface growth was tested by taking duplicate samples, and the following figures give the number in two separate samples for both the "exposed" and "sheltered" areas during the winter months:

Exposed area		Sheltered area	
Sample 1	Sample 2	Sample 1	Sample 2
28	14	460	590
74	44	162	177
41	68	326	291
0	1	181	174
510	790	402	290
90	95	387	315
128	139	236	234
2	1	—	—
47	44	—	—
130	135	—	—

In summer the worms are much more abundant, and the numbers in four separate samples of alga, taken on two different occasions, were:

	Sample 1	Sample 2	Sample 3	Sample 4
6 Aug.	1750	1750	2000	1750
7 Aug.	1500	1750	2250	2000

The reliability of the method used for the 12 and 30 in. levels was tested by using two bags for one tube on four occasions. The numbers are given below:

Sample 1	Sample 2
2600	2350
1750	1400
1400	1150
2000	2000

The agreement shown is quite reasonable, and the average gives a fair estimate of worm abundance. The close similarity in the fluctuations for the "exposed" and "sheltered" areas, both in worm numbers and quantity of alga, indicates that the sampling is fair (see Text-fig. 1).

Some idea of the movement of the worms into the bags was obtained by having two bags in one pipe, one of which remained in position the whole week while the other was replaced each day. It was found that the number migrating into the bag from day to day fluctuated greatly but with a certain regularity in that a large entry generally preceded a small entry on the following day, and conversely. With one exception a constant ratio of 7 to 2 was found between the sum of the daily accumulations and the weekly accumulation:

Week	Week's aggregate of worms in bag changed daily	Number of worms in unchanged bag
1	4300	1400
2	2200	700
3	2300	600
4	7000	2200
5	2100	2000
6	3100	900
7	7700	2400

The evidence given by having two bags in one tube for the same period and the demonstration of a balance between the number of worms entering the bags and the number in the bed at that particular depth (ratio of 7 to 2) indicates that the method is representative. Any depletion of worms by their constant removal in the bags must be negligible because of their great abundance and their active migration locally.

Description of the surface growth of *Phormidium* and its seasonal changes

Description of the surface growth

The surface growth of alga consists of a thick covering of *Phormidium* (Cyanophyceae) which is distinguished from *Oscillatoria*, also found on the beds, by the much more compact nature of the growth due to agglutination of the filaments. Quick growth of these filaments accompanied by

their rapid formation enables a considerable stratum to form in a relatively short time.

A definite cycle of growth takes place from one sloughing to the next. The first stage in the recolonization of a clean pebble (Pl. IX, fig. 1) is the spreading of a thin layer of filaments from a number of centres which join up to form a continuous layer. This gradually becomes thicker (up to 2–3 mm.) and assumes a leathery condition (Pl. IX, fig. 2). The appearance of nodules, for the most part solid, has been commonly observed at this stage, and at these places the sheet ruptures and the edges curl away. The smooth layer may also be destroyed by weathering, particularly excessive drying-off and wind action, giving rise to ragged, curled edges. The sum total of these forces together with the depredations of insect larvae, worms and nematodes reduces the quantity of the growth (Pl. IX, fig. 4). One further important change takes place, particularly during the warmer months of the year. Part of the alga assumes what has been termed the "spongy" condition. In this state the growth loses its compact, leathery nature, becoming very soft with an irregular surface in contrast to its former smoothness (Pl. IX, fig. 3).

Seasonal changes of the surface growth

During the winter months in which these observations were commenced (January–March 1937) the surface growth remained leathery and generally firmly attached to the pebbles. The presence of incomplete patches with the edges curling away showed the effects of weathering and the activities of the scouring organisms. A few of the pebbles were clean or had a new growth forming. In March there was a slight decrease in quantity of the thick growth with an increase of clean pebbles.

Early in April a distinct change was observed, the alga had become extremely soft and spongy and riddled with worms. A few days later it was entirely spongy and lying loosely on the pebbles, a living mass of worms and impalpable debris. This condition prevailed until early in May, when it was apparent that a considerable reduction in quantity of growth had occurred. The reduction continued until, a few days later, the pebbles were either clean or had only small patches adhering. This change is called the off-loading of the surface growth and is characterized by the rapid onset of sponginess and rapid reduction in quantity. The worms also left the surface, since there was little to feed upon or to conceal them.

During this period of scarcity of *Phormidium*, a bright green covering of *Ulothrix* (Isokontae) and *Chlorella* (Isokontae) appeared on the pebbles.

An obvious new growth of *Phormidium* commenced at the end of May, rapidly replacing the other algae, and early in June a thin sheet was covering the medium. A thick continuous sheet was formed on most of the pebbles by the middle of this month. Throughout June and the first half of July the growth remained for the most part leathery, but was beginning to show the effects of weathering. In the latter part of this month, when the worms had again increased, areas of spongy alga coincident with worm aggregations were noticed. During the summer and autumn, and as late as November, the alga fluctuated continually between the spongy and leathery conditions, remaining bulky all the time. These changes sometimes took place rapidly, so that the pitted surface of the growth gave the impression of sponginess, whereas it was quite firm. The appearance of the spongy condition, without exception, coincided with the presence of large numbers of worms in the growth, and the leathery condition with their scarcity. The degree of dominance of one condition over the other seemed to be determined by the length of time the worms remained in the growth. One important point concerning the sponginess of the alga during the summer is that in a large number of cases it was of a more superficial nature, and the base of the sheets remained adherent to the substratum instead of lying loosely upon it. In the later weeks of October the alga had become completely spongy around the edges of the pebbles, and this was washed off leaving a thick central capping.

During the ensuing months (November–January 1937–8) the alga remained unchanged. The first indication of sponginess was seen in the third week of January, at the time when the worms first became abundant in the growth. The alga was almost entirely spongy and riddled with worms by the second week of February (2600 worms/sq. ft.). This early appearance of the worms *en masse* and the subsequent off-loading can be accounted for by the mild weather experienced in the early months of this particular year (1938) which precipitated events. During the next fortnight considerable reduction in quantity took place comparable with that in April of the previous year. (In the graph (Text-fig. 1) showing the numbers of worms in the alga, this rapid increase is masked by the low numbers which followed it.) The alga remained scarce until the end of the first week in March, when regrowth was so rapid that a fairly thick stratum was formed a fortnight later. During April the growth gradually became spongy again in certain areas where the worms had increased.

The growth in the "sheltered" area underwent similar changes but was consistently less in quantity, probably partly due to the shorter period of sunlight through interference by the wall.

The quantity of growth is shown in Text-fig. 1; for March 1938 two readings are given because two distinct phases occur in this month, namely, the end of the off-loading and the spreading of a new growth. It will be noted that the maximum dry weight sq. ft. was 8·4 g. and occurred in August, while the minimum was 1·7 g. ("exposed" areas) occurring in May 1937.

The abundance and seasonal distribution of the Enchytraeid worms

Factors influencing the abundance of the worms

The striking abundance of the worms indicates that these particular bacteria beds afford an especially favourable environment for them. A number of factors probably contribute to this: first, the medium is always covered by a film of sewage and, even when the beds are rested for a few days, only the upper few inches dry out, so that the worms are protected against desiccation. The temperature of the beds is more favourable than some of the natural habitats (Stephenson, 1930), with the result that in the winter months the vital activities of the worm, particularly breeding which occurs mainly from November to May, are much enhanced. Observations have shown that the production of cocoons, rate of development and viability are adversely affected by temperatures below 6° C., and in only 1 month in the period of 5 years has the mean monthly temperature in the bed fallen below this critical point.

The sewage continually brings a supply of food in the form of suspended solids, which is further augmented by the surface growth and fungal growths in the depths. There are no typically carnivorous organisms common in the beds likely to feed upon the worms, and predation in general seems to be at a low level. They are, however, exposed to the attacks of various birds, especially starlings and meadow pipits, when they occur in the surface growth.

The absence of any drastic chemical treatment of the sewage at Knostrop before it is conveyed on to the beds is also an important point affecting the survival and increase of the worms.

Seasonal distribution of the worms

The abundance of the worms in the surface growth, in the depths, and in the bed effluent has a seasonal variation. The fluctuations of the number in the surface growth during the winter months show a definite correlation with temperature changes, which is sometimes modified by

resting of the beds. From a study of the weekly variations and by means of experiment it has been shown that the worms migrate from cold. The distribution of large numbers of worms in columns of pebbles with a graded temperature was observed, and it was found that they moved downwards from the surface when this was chilled to 5–6° C.

Throughout the cold months the worms remain relatively scarce in the growth, but immediately the temperature rises in spring they migrate into it in very great numbers, and so long as the growth remains sufficiently thick are abundant until the temperature falls again. The worms are generally more abundant in the "sheltered" area in winter, which is almost certainly due to the higher temperature prevailing there. For only one short period in March 1937 did the reverse occur, and at this time an easterly wind was blowing equalizing the temperature of the two areas. The number of worms in the surface growth varies greatly in summer. This is not caused by temperature changes but is due to the bed drying off while it is being rested. It is less apparent in winter owing to the much decreased rate of drying.

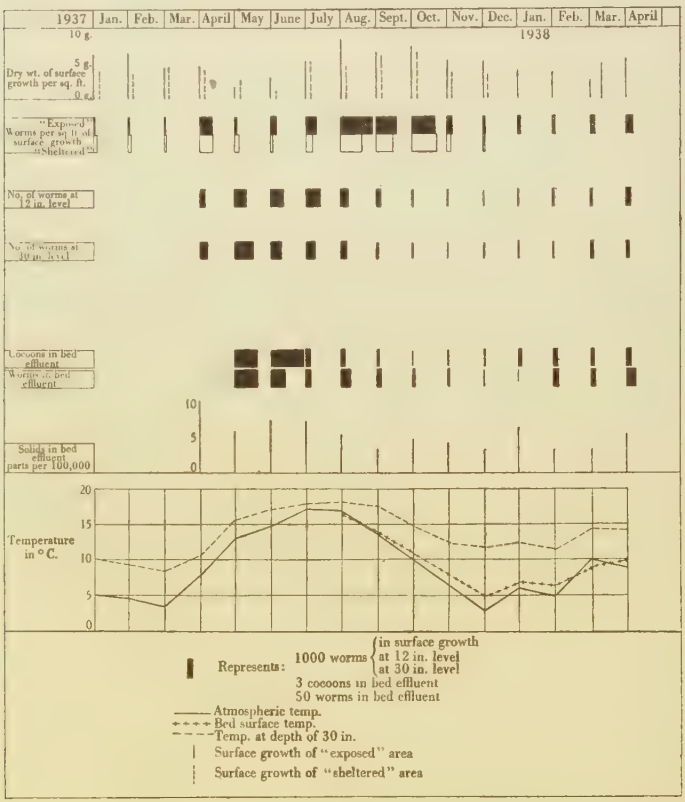
Seasonal variations are also shown to occur at the 12 and 30 in. levels; here again the worms are scarce in winter becoming abundant during the spring, but they decline somewhat in summer when the worms are most numerous at the surface (Text-fig. 1). There can be no temperature control at these depths such as occurs at the surface, since the lowest average temperature is 9° C. which does not affect the worms adversely; in fact, this temperature at the surface in winter attracts the worms. The temperature fall in winter might reduce their activity and so alter the rate of entry into the bags of food, but this seems improbable, since observations of the worms at temperatures ranging from 3 to 25° C. have shown that it is only below 5–6° C. that activity is reduced appreciably. This agrees with Welch's work (1914) on the effect of temperature on worm activity. Further, the relative scarcity of the worms at these depths in summer must be accounted for.

A relative abundance of worms and cocoons in the bed effluent is confined to the spring months of the year. The temperature of the bed is uniform, as is shown by the fact that the temperatures of the tank and bed effluents are found to be very similar. This again discounts the presence of any temperature influence operating at the bottom of the bed.

It would appear from Text-fig. 1 or Table I that a considerable absolute reduction in worm population occurs in winter. That this is more apparent than real and due to the relative scarcity from the regions tested is shown by the fact that during the winter and early spring, whenever

the temperature allowed, the worms became abundant at once in the surface growth. This statement is further supported by the observations of their concentration in the upper 6 in. which showed that they remain abundant here throughout, only receding below 3–4 in. at very low temperatures.

Text-fig. 1 is a graphic picture of the relative distribution of the worms in the bed at various levels, on a depth scale during the period covered. The quantity of alga on the surface is also shown, together with the solids issuing in the final effluent. A temperature scale for the surface and the 30 in. level is shown below.



Text-fig. 1. The seasonal abundance and distribution of the worms in the bed on a depth scale, the quantity of surface growth, solids in bed effluent and temperatures, in monthly averages.

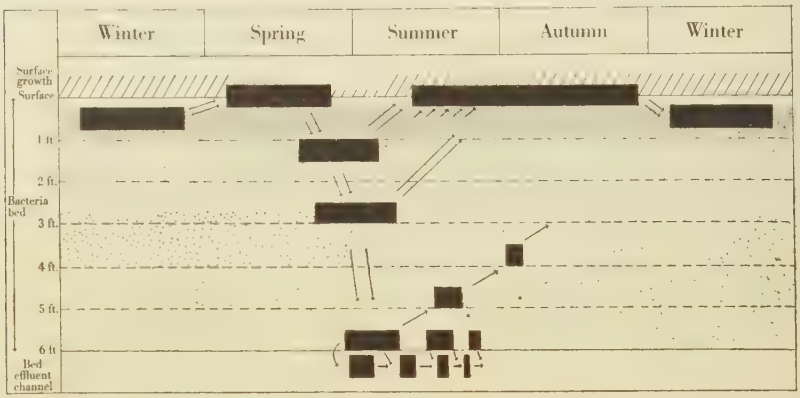
Table I. *Number of worms at the surface, 12 in. level, 30 in. level and in the bed effluent, the number of cocoons in the bed effluent, the quantity of surface growth and the bed temperatures. The figures for each month were obtained by averaging the weekly estimates*

	No. of worms/sq. ft. in the surface growth		No. of worms in bags of food		No. in weekly strained sample of bed effluent		Dry wt. in g. of surface growth/sq. ft.		Mean temperatures in ° C.		
	Exposed	Sheltered	12 in. level	30 in. level	Worms	Cocoons	Exposed	Sheltered	Atmosphere	Surface	30 in.
1937											
Jan.	300	600	—	—	—	—	6·3	3·8	4·9	—	10·1
Feb.	300	550	—	—	—	—	6·4	3·3	4·5	—	9·3
Mar.	200	400	—	—	—	—	4·6	4·5	2·5	—	8·5
Apr.	2500	2700	1000	1600	—	—	4·6	4·1	7·8	—	10·7
May	500	900	2500	4000	250	14·0	1·7	2·5	13·0	—	15·3
June	1000	400	2600	2400	160	21·0	2·9	1·2	14·75	—	17·0
July	2300	1400	3000	2000	60	3·3	5·4	5·4	17·75	—	18·0
Aug.	6600	4450	1450	1200	100	2·25	8·4	4·7	17·0	16·4	18·0
Sept.	4200	3400	800	400	20	1·0	6·5	6·0	13·3	13·9	17·8
Oct.	4950	5200	200	200	20	0·25	7·8	6·5	10·2	11·5	15·1
Nov.	1200	800	100	100	10	0·33	5·6	3·6	6·3	8·0	12·0
Dec.	400	300	50	100	5	0·75	5·5	3·3	3·5	4·6	9·0
1938											
Jan.	550	—	100	200	1	1·5	4·1	—	5·7	7·1	9·8
Feb.	600	—	200	200	30	3·25	3·9	—	5·2	6·4	9·0
Mar.	900	—	400	600	30	1·4	5·2	—	9·7	9·3	12·2
Apr.	1500	—	900	850	50	2·25	5·7	—	8·7	9·8	11·9

From these data it has been possible to construct a theory of the seasonal migrations of the worms. It is to be understood that the worms are scattered throughout the bed, but they concentrate at certain levels, and it is these concentrations which are referred to in the following account. During the winter months the worms remain scarce on the surface, the 12 and 30 in. levels, and also in the bed effluent, the bulk remaining in a layer from the surface to a depth of approximately 8 in. Immediately the temperature permits in spring they migrate into the surface growth in large numbers. Meanwhile a slight increase takes place in the depths. Soon after this movement into the alga the surface growth off-loads, and a new growth commences, the two processes occupying about 6 weeks, and during this time the worms are scarce at the surface due to the scarcity of food and concealment, but they increase greatly at 12 and 30 in.

The alga which has been washed from the surface, together with the material from the general off-loading of the medium, is slowly carried down the bed, followed by the worms and insect larvae which feed on it, causing an increase in their numbers lower in the bed. Some of the worms and larvae continue to migrate with the alga to the bottom of the bed and, as a result, a proportion of them are washed out in the bed effluent. This explanation receives strong support from observations of

Graham (unpublished) upon the relative abundance of larvae and pupae in the same bed effluent, contrasted with the number of larvae in the bed and the number of flies emerging from the bed. He finds that during the off-loading period considerably greater numbers of larvae and pupae occur in the bed effluent, and this is not solely due to increased numbers in the bed, but must be caused by a downward migration such as is postulated for the worms. In early summer, as soon as a considerable new growth has re-formed at the surface, the worms move up into this fresh source of food. It is also very probable that an upward migration from the depths, shown by the reduction of the worms at 12 and 30 in. (with no increase in the bed effluent), is accentuated by a distinct shortage



Text-fig. 2. A theoretical representation of the seasonal mass movements of the worms in the bed. General abundance in the bed is shown by density of stippling, and concentrations by rectangles.

of food in the depths at this season owing to the enormous increase of fly production. The concentration of worms remains at the surface in the growth during the remainder of the warm months, finally being driven below by the onset of cold weather. It does not reach a depth of 12 in. however, descending no more than necessary to avoid the cold.

The worms breed during the whole year, but this activity is greatest in the winter months. No influence of this factor on general abundance can be detected, and this contrasts remarkably with the behaviour of the various flies present (Lloyd, 1937). It is interesting to note that Welch (1914), studying a very similar case, found variation in abundance in the upper layers and bed effluent which would agree with this theory, although he does not offer any explanation. A diagrammatic representation of these mass movements is given in Text-fig. 2.

The influence of the worms on the condition of the surface growth

The effect of the worms on the character of the growth

It has been mentioned previously that the appearance of the spongy type of growth on the beds always coincided with the presence of large numbers of worms in it, and that the spring off-loading closely follows the spring migration of worms into the alga. Insect larvae are so scarce in the alga that their effect on surface growth can be ignored.

Examination of the growth showed that over the whole bed areas of spongy alga coincided with areas where the worms were very abundant, and that even in patches on single pebbles the same connexion was evident. Microscopic examinations of both the spongy and healthy types, collected from the same area, have been made on a number of occasions at different times of the year, including the off-loading periods. It was found that a greater accumulation of greenish brown granular worm faeces occurred in the spongy type of growth. There was no morphological difference in the filaments of the alga. An indication that the filaments of the healthy growth were slightly more vigorous than those of the spongy growth was given by the use of neutral red, which appeared to stain the protoplasm of the latter to some extent (Guilliermond, 1934). Protozoa were never present in quantity. Nematodes were fairly numerous in a few of the samples of both the healthy and spongy growth but showed no consistent correlation with any particular condition. This evidence suggests strongly that the worms are responsible for the degeneration of the growth.

To facilitate a study of the alga in the absence of the worms, pebbles were isolated in shallow pot trays, $10 \times 7 \times 2$ in., placed inside shallower larger trays, so that there was a moat $\frac{3}{4}$ in. wide between them. These trays were set into the bed, level with the surface, and the moat and inner tray filled with tank effluent as the distributing machines passed over them. Muslin bags containing copper sulphate crystals were placed in the moat twice weekly in winter and every day in spring. Three couples of such trays were used containing large, smooth pebbles with incipient growth on them, surrounded by smaller pebbles (P. IX, figs. 7, 8). The experiment was commenced in September 1937, and by the end of October a thick, smooth layer of alga had formed over each pebble. Later nodules appeared in the growth on some of the pebbles and these, together with the effects of weathering, resulted in the alga being torn away to some extent around the edges. The growth continued in this

healthy condition during the winter months. When the movement of worms into the surface growth took place towards the end of January and early in February, and the alga on the beds became generally spongy, that on the isolated pebbles remained unchanged, and during the subsequent off-loading it still retained its thick leathery growth. At this time it would have been impossible to choose five pebbles (the number in the trays) at random over the bed which had this type of covering, and this clearly indicates the significance of the test. As a final experiment the worms were introduced into one of the trays in March, and after 3 days the alga in this tray was becoming spongy where the worms had collected. These areas continued to spread (Pl. IX, fig. 6), and a fortnight after the introduction of the worms the alga was completely spongy. During this time the growth on the pebbles in the other trays did not alter.

This last evidence finally proves that the worms are responsible for the spongy character of the growth. The softness and sponginess is due to the worms feeding on it and its gradual conversion into faeces. In the advanced stage the worms seem to plough it, giving rise to the uneven, pitted surface.

The influence of the worms on the off-loading of the surface growth

That the off-loading of the algal growth occurs soon (14 days) after the spring invasion of worms is very significant, and there seems to be a definite connexion between the change to the spongy type of growth and the off-loading at this time. Further, no evidence of any other cause could be found. Drying of the bed surface causes no sponginess but a kind of peeling which results in discrete pieces of growth being dislodged and washed down amongst the pebbles, as later happened to the growth in one of the trays mentioned above; complete off-loading cannot, however, be accounted for in this manner. The absence of off-loading on the isolated pebbles at a time when it was taking place generally on the bed is important.

The main obstacle to this theory is that in summer when the worms are abundant in the growth and sponginess of the latter is common, a general off-loading does not occur. However, a reasonable explanation of this may be that during summer the algal growth tends to keep pace with the depredations of the worms. It is well known that the rate of growth of blue-green algae is increased by higher temperature and longer light (Hesse *et al.* 1937). Some idea of the high rate of growth on the beds in summer is given by the fact that when two selected areas were scraped

clean a new growth equivalent to 5·2 g. (dry weight)/sq. ft. re-formed in a month. Attachment to the substratum is often maintained during "summer sponginess", and it is probable that this is less firm in spring after the growth has withstood the weathering effects of winter, and so is more likely to off-load at this time. The autumn off-loading, which has been reported to occur sometimes, can be explained in a similar manner to the spring off-loading, the regeneration of the alga diminishing as the days shorten.

It is interesting to note that the severity of the off-loading in February 1938 was decidedly less than that of the previous year, and also that the attack of the worms was much less prolonged on this occasion due to a following cold spell of weather.

All this evidence clearly indicates a close connexion between temperature, season, and severity of worm attack as precipitating an off-loading of the surface growth.

PART II. THE INFLUENCE OF *LUMBRICILLUS LINEATUS* MULL. ON THE EFFICIENCY OF BACTERIA BEDS

Introduction

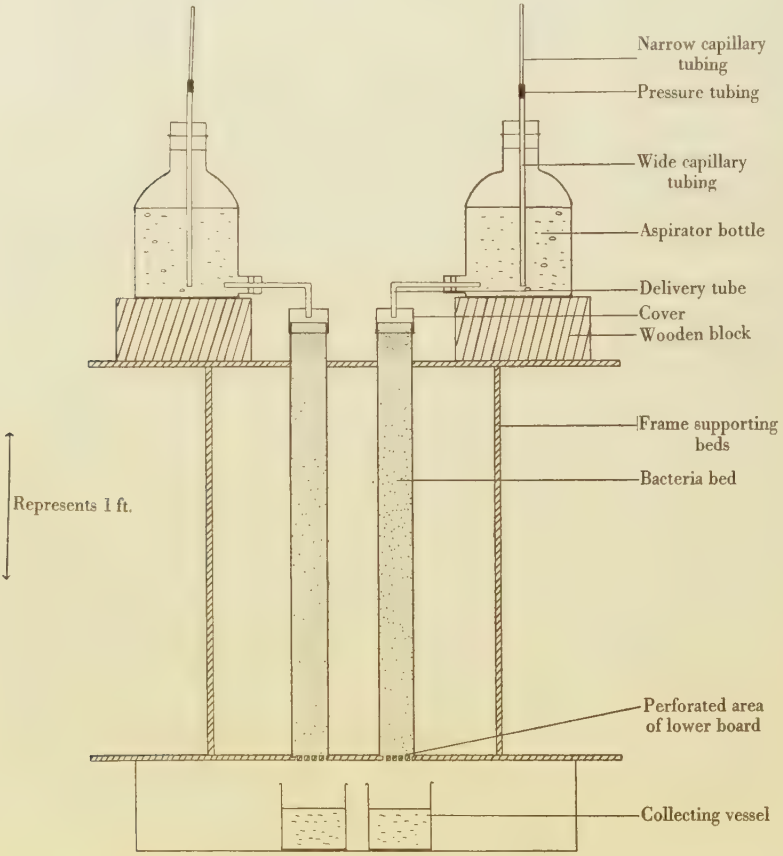
The scouring action of organisms in the bacteria bed has been emphasized in Part I, and also the special significance of the Enchytraeid worms in this connexion, particularly the relationship between them and the off-loading of the surface growth. The activity below the surface is not so obvious, and it was necessary to assess the part played there by means of laboratory tests. Model bacteria beds were therefore constructed, and the scouring capacity of the worms was studied under controlled conditions. Investigations of a similar kind but in relation to the Collembolan *Achorutes viaticus* Tulb. were carried out by Parkinson & Bell (1919). They found that a bed inoculated with this insect remained open and delivered effluent of a high quality with a good degree of nitrification, whilst a bed without a scouring organism became choked and useless.

Description of apparatus

The experiment was carried out in a photographic dark room to reproduce as near as possible the conditions in the bed where only the upper surface is exposed to light.

Two glass cylinders, each 36 in. long and 3 in. in diameter, filled with marble chippings as a medium, functioned as bacteria beds and were supported side by side in a vertical position by a wooden frame (Text-fig. 3). The cylinders fitted circular depressions cut into a board upon which their lower ends rested. These depressions

were perforated by eight holes each of $\frac{1}{4}$ in. diameter, through which the liquid trickling down the bed could drain away into a sink over which the apparatus was fixed. The tank effluent was delivered on to the beds from two large aspirator bottles placed above the cylinders. The marble chippings used for the medium averaged about $\frac{1}{2}$ in. diameter and were packed evenly into the cylinders. The topmost 3 in. of the



Text-fig. 3. Diagram of model bacteria beds.

medium consisted of chippings averaging $\frac{1}{4}$ in. diameter to spread the effluent evenly. Covers of bolting silk, supported in a cardboard frame into which the delivery tube from the bottle closely fitted, were kept over the tops of the glass cylinders. The portion of the sink used in the experiment was also screened off. These precautions were necessary to keep out the sewage flies. A barrier of bolting silk was placed between the two drainage areas of the beds after the introduction of the worms to prevent their migration from one cylinder to the other. It was possible to arrange a constant rate of

flow of any desired volume by constructing a hydrostatic head for each bottle. This entailed the use of capillary tubing with a bore 0·3 mm. in diameter, the rate of delivery being determined by the length of tubing used (Jenkins, 1933). This narrow capillary was attached to wider tubing which passed through the tightly fitting rubber bung of the bottle.

METHODS

The working of the model beds

The experiment was continued for a period of 23 weeks, from October 1937 until March 1938. Four and a half litres of tank effluent were delivered on to each bed daily, representing a rate of flow of 80,000 gal./acre/hr. The beds were worked for 12 hr. in the day, 6 days in the week and rested the remaining day. The tank effluent was heated up to 50° C. and cooled before use, as a precautionary measure against the presence of fly larvae and eggs. During the first 10 weeks crude sewage was used, but later it was strained through cotton-wool which removed the bulk of the coarser suspended solids and lightened the task of purification.

Chemical analysis of tank and bed effluents

Chemical analyses of the tank and bed effluents were carried out twice a week, and included determinations of the following according to the methods outlined in *The Standard Methods of Water and Sewage Analysis* (H.M.S.O. 1928):

(1) Free and saline ammonia, for both effluents.
(2) Albuminoid ammonia, for both effluents.
(3) Oxygen absorbed in 4 hr. from potassium permanganate, for both effluents.
(4) Nitrites in bed effluent.
(5) Nitrates in bed effluent.
(6) Suspended solids in bed effluent.

The samples were collected in beakers placed under each drainage area on the previous evening, but for the suspended solids a collecting vessel was used over the whole period between sampling. The condition of both beds, as far as could be determined, was noted from time to time.

They were worked for 5 weeks before the worms were introduced into bed A to make certain that both beds were functioning in a reasonably similar manner, and also to mature them before supplying the worms. On the eighteenth week worms were also added to bed B.

OBSERVATIONS OF THE BEDS

In both of the beds the medium was heavily coated with black deposits at the end of the third week, and these had become very heavy by the fourth week. During the next week approximately 1500 worms were added to bed A. They soon spread, and cocoons became common on the glass walls and medium. It was noticed by the tenth week (sixth after introduction) that the worms were reducing the areas of dense deposit. The following week an increase of solids began to appear in the effluent and soon became very heavy. This continued until about the

fifteenth week, the medium becoming gradually cleaner, until only slight deposits were present on the walls and marble chippings. All this time the solids continued to accumulate in the other bed until the medium could hardly be discerned, and black foul areas appeared. On the fourteenth week this bed was choked up and severe "ponding" occurred, i.e. the liquid was unable to drain through and collected on the surface. This meant that the bed was out of action and had to be rested for 5 days, but soon ponded again.

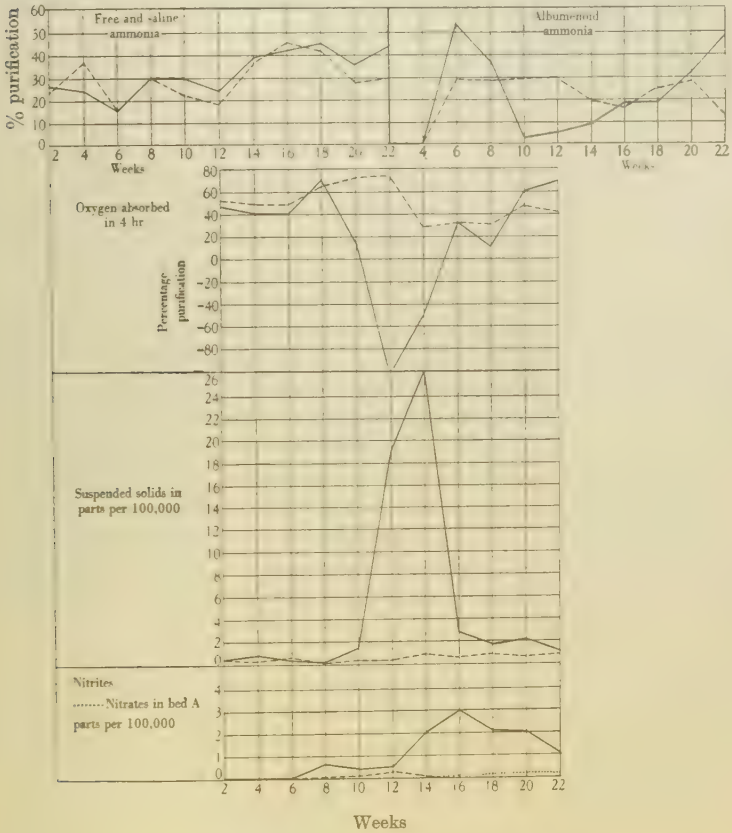
About this time there was a marked difference in the bed effluents, that from bed A was bright and clear as tap water, while that from bed B was milky and little different from the tank effluent.

On the eighteenth week worms were added to bed B to see if they would clean the medium as they had done in bed A. By the twentieth week they had spread uniformly in the bed, as far as could be determined, but, in the next few days, they all died in the bottom two-thirds of the cylinder. Worms were supplied on two further occasions, but each time they died, and on the last the whole bed became foul, so the attempt was abandoned. It seemed evident that this bed had become too septic for the worms to live in it for any length of time. Meanwhile they had continued to multiply in bed A. It was noticeable that after the introduction of the worms into bed B, it had become more porous so that no further sign of ponding had occurred.

Results from the chemical analysis of the tank and bed effluents

The results, apart from the nitrites, nitrates and solids, are expressed as a percentage purification of the tank effluent by an average of the four analyses made over a period of 14 days (Text-fig. 4). Early analyses of the effluents from the two beds indicated that initial purification, as shown by the tests of free and saline ammonia and oxygen absorption, was slightly better in bed B, so the worms were introduced into the less efficient bed, namely, A. Two well-defined phases occurred in the bed containing the worms: first, the washing out of the solids from the bed after they had been loosened and broken up by the worms. This had an initial detrimental effect on the character of the bed effluent as shown by the estimations of albuminoid ammonia, and the oxygen absorbed, which was due to the solids present as analyses of the decanted liquid proved. The second phase covers the gradual recovery of this bed until a steady high efficiency was being maintained. The quantity of free and saline

ammonia is not affected by the amount of solids in the bed effluent, and this test shows a gradual increase in the standard of purification, especially in bed A. On the other hand, the control bed shows a steady,



Text-fig. 4. Results of the chemical analyses of the two bed effluents.
—— Bed A. – – – – Bed B.

moderate efficiency over the first period, with a subsequent decrease, so that the degree of purification carried out in the two beds becomes very different. It seems probable that the presence of the dead worms in bed B in the last 2 weeks of the experiment increased the amount of albuminoid ammonia in the effluent from this bed.

The outstanding contrasts in the behaviour of the two beds are:

(1) The heavy off-loading of the solids in bed A caused by the worms, and their gradual accumulation in bed B, which was eventually choked.

(2) The active nitrification in bed A, in contrast to its virtual absence in bed B.

This last activity takes place only when the putrescible matter in sewage has been removed, and is often taken as a measure of the efficiency of a bed (Buswell, 1928).

The essential difference in the character of the bed effluents is further shown by the methylene blue test for stability. This was carried out over the last 8 weeks of the experiment. The effluent from bed A proved stable in thirteen out of sixteen tests, while that from bed B was putrescent in every case.

DISCUSSION OF PARTS I AND II

Observations recorded in this paper, together with those from bacteria beds in general, leave little doubt that the off-loading of the zoogloeal growth and accumulated solids, which occurs in the bed during the spring and sometimes in the autumn, is the result of biological activity.

Parkinson & Bell (1919) have shown that *Achorutes viaticus* (Collembola) is capable of causing it, and now the ability of *Lumbricillus lineatus* (Enchytraeidae) to bring about these changes has been demonstrated. Further, there appears to be no reason why other organisms of similar habits, living in the bed, should not behave in the same way. Actually the larvae of *Psychoda cinerea* Banks (*P. compar* Eat.) cleaned the medium of the model bacteria beds used in these experiments, when introduced accidentally, with the result that the preparation of the beds for the test on the activity of the worms had to be started afresh with the addition of certain precautionary measures mentioned above, such as the heating of the sewage to 50° C.

This spring phenomenon can be explained by the gradual stimulation of the bed fauna through the rise in temperature, but a more exact explanation can be given for the beds described in these experiments (see also Part I). Here the surface growth of *Phormidium* (Cyanophyceae) is slowly washed down into the bed in spring after being loosened, mainly through the greatly increased number of worms feeding on it. The worms and larvae continue to feed on this detached growth, following it into the depths of the bed, where they also attack and loosen the zoogloeal growth around the pebbles. In consequence, a general off-loading of the bed is precipitated. A further important feature of the activity of these

worms is that they also break up the growth and prevent its accumulation in the upper layers of the bed, which would otherwise become choked. The prevention of such choking in the bacteria bed still remains one of the outstanding problems of this widely used system of sewage treatment (Jenks, 1937). Hence the presence of the worms is essential if these beds are to remain open. Other forms, such as insect larvae and Nematodes, are also important in helping to break up the growth, but work mainly below the surface.

The fauna of bacteria beds varies with the sewage works according to local conditions and different organisms are dominant, but they perform this same function. At Huddersfield, for example, the surface growth and worms are absent, and here, presumably, the abundant larvae of *Psychoda alternata* Say. perform this cleansing of the bed (Golightly, unpublished). These beds are treated periodically with creosote to keep down the flies (Scouller & Goldthorpe, 1932), and this probably accounts for the absence of worms.

The impression given so far is that the action of these larger forms is solely beneficial. This is not strictly true as, occasionally, chemical treatment of the sewage is necessary to keep down the emerging flies. The need for this is due to the frequent imperfection of the biological balance which hinders seriously the work of purification. No information has been obtained upon the most desirable combination of organisms in the fauna for the production of the most efficient treatment of sewage by the bacteria bed method. Too little is known of the interrelationships of the bed faunas, and especially their ability to check or compete with one another under changing conditions. For instance, *P. alternata* and *Metriocnemus longitarsus* Goet. increase periodically and are a nuisance to nearby dwellings and to those employed on the beds, but their larvae are beneficial. Again, various worms and larvae are washed out of the beds into the final settling tanks, often in large numbers, where they decompose and cause a serious deterioration of the final effluent. The small Enchytraeid worms and the larvae are not so important in this respect as the bulky Lumbricid worms, and it seems probable that the harm done by these large earthworms is far in excess of any good they might accomplish, and hence they are undesirable.

Welch (1914) carried out experiments to determine the effect of *Lumbricillus rutilus* Welch (probably *L. lineatus*) on the putrescibility of sewage by enclosing the worms in stoppered bottles containing sewage, with a series of controls containing sewage alone. He found that the former became putrescible first, owing to the effects of the respiration of the

worms and the accumulation of their excreta, so that to this extent he considered the worms harmful. But it has been pointed out (Part I, p. 142) that the worms are not immersed in the sewage in the bacteria bed, but take their oxygen from the air circulating in the bed. Further, the experiment with the model beds suggests that the consumption of atmospheric oxygen, the presence of the worm's excreta, and the dead bodies, in a healthy bed, do not have any serious ill effect. Therefore, it is concluded that the Enchytraeid worms are on the whole beneficial, and of particular importance under the conditions obtaining at Leeds.

Only the mechanical effects of these macro-organisms have been thought important, and the involved biochemical changes taking place in the bacteria bed, with the ultimate production of nitrate from proteins, have been considered to be due to bacterial action alone. The introduction of *Achorutes viaticus* (Collembola) and *Lumbricillus lineatus* into experimental beds leads to increased nitrification of the effluent, and this may be due to an indirect influence as suggested by Dyson & Lloyd (1933), or, on the other hand, the chemical changes of metabolic activity in both Protozoa and Metazoa present may produce considerable effect in a more direct manner upon the efficiency of the treatment. Physiological studies of the organisms in the bed are essential therefore to a full appreciation of their influence.

SUMMARY OF PARTS I AND II

1. The flora and fauna of the bacteria bed of a sewage plant are described, and their interrelationships discussed.

2. A definite cycle in the character and quantity of the *Phormidium* (Cyanophyceae), the main constituent of the surface growth, is described, the most striking feature of the cycle being the spring off-loading.

3. The Enchytraeid worm, *Lumbricillus lineatus* Mull., is briefly described and its habits observed in relation to the conditions in the bed. The worms feed primarily on the growth of *Phormidium*, and consequently tend to cluster at the surface, when this is abundant and climatic conditions allow. Cold drives them slightly down, but the off-loading sends them deeply into the bed, when an increased proportion pass out with the effluent. Drying of the surface in summer sends them down in an erratic manner according to the degree of desiccation.

4. The cause of the off-loading of the surface growth in spring is its change from a leathery to a spongy condition. The worms are mainly responsible for this and, therefore, for the off-loading. The alga rapidly

re-forms (3-4 weeks) upon the pebbles, worms migrating into it again when it is sufficiently thick. The absence of an off-loading in summer when the growth is again spongy is attributed to the higher rate of regeneration owing to increased temperature and sunlight, which lessens the effect of the worm depredations.

5. The importance of *Lumbricillus lineatus* in keeping open a bacteria bed and helping to maintain its efficiency is proved by means of experimental beds. Worms introduced into an inefficient bed with solids rapidly accumulating cleared the medium and allowed active nitrification to commence. If the bed is very badly choked and septic, the worms may be unable to carry out this function. It is probable that all the larger scouring organisms abundant in the bed have this property to a greater or lesser extent.

6. The mechanism of the spring off-loading is discussed with special reference to the role of *L. lineatus* in the Leeds bacteria beds.

7. The importance of the maintenance of the biological balance by means of the most economic fauna is pointed out.

8. Finally, the need for more intensive physiological studies is stressed.

It is a pleasure to acknowledge the patient help and invaluable advice of Dr Ll. Lloyd during this work. Thanks are due to Mr J. T. Thompson, Manager of the Leeds Sewage Works, who has been a great aid throughout, especially on technical details, and also to Mr J. F. Graham who is responsible for the photographs in the paper, and who supplied the material for estimating the worms in the bed effluent. Prof. E. A. Spaul has kindly read and discussed the paper. The writer is indebted to the Department of Scientific and Industrial Research for the grant which has permitted this study to be made.

REFERENCES

Beddard, W. (1895). *Monograph of the Oligochaeta.* Oxford.

Buswell, A. M. (1928). *The Chemistry of Water and Sewage Treatment.* New York.

Dyson, J. B. & Lloyd, Ll. (1933). Remarks on the flies breeding in the bacteria beds at the Knostrop Sewage Works, Leeds. *Proc. Inst. Sewage Purif.* Pt. 2.

Guilliermond (1934). Le Vacuôme ou Système Vacuolaire. *Actualités Scientifiques.* Paris.

Hesse, R., Allee, W. C. & Schmidt, K. P. (1937). *Ecological Animal Geography.* London.

Jenkins, S. H. (1933). The design of experimental percolating filters. *Biochem. J.* **27**, 240.

Jenks, H. N. (1937). The renaissance of the percolating filter. *Proc. Inst. Sewage Purif.* Pt. 1.

LLOYD, LL. (1935). The bacteria beds of sewage works as an environment for insects. *Proc. roy. Soc.* **10**, 34.

—— (1937). Observations on sewage flies; their seasonal incidence and abundance. *Proc. Inst. Sewage Purif.* Pt. 1.

MICHAELSEN, W. (1900). *Das Tierreich. Oligochaeta.* Berlin.

PARKINSON, W. H. & BELL, H. D. (1919). *Insect Life in Sewage Filters.* Sanit. Pub. Co.

RUDOLFS, W. (1924). Film removal studies on biology of sewage disposal. *Bull. N.J. agric. Exp. Sta.* no. 403.

SCOULLER, W. D. & GOLDTHORPE, H. H. (1932). Control of the sewage filter fly (*Psychoda*). *Publ. Hlth Congr.* 1932.

STEPHENSON, J. (1922). On some Scottish Oligochaeta with a note on encystment in a common fresh-water Oligochaete, *Lumbriculus variegatus* Mull. *Trans. roy. Soc. Edinb.* **53**, 2.

—— (1930). *The Oligochaeta.* Oxford.

WELCH, P. S. (1914). Studies on the Enchytraeidae of North America. *Bull. Ill. Lab. nat. Hist.* no. 10.

EXPLANATION OF PLATE IX

Figs. 1–4. Different types of surface growth.

Fig. 1. Clean pebble after off-loading.

Fig. 2. Firm healthy growth cut to indicate thickness.

Fig. 3. Spongy type of growth.

Fig. 4. Growth illustrating the effect of weathering and attacks of worms and other organisms.

Figs. 5–8. Pebbles isolated in the trays and the effects of the worms on the growth.

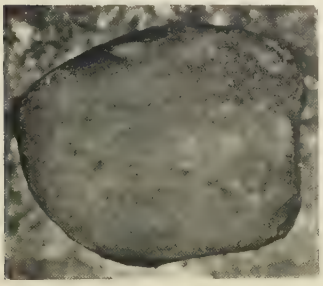
Fig. 5. Growth of control at end of experiment.

Fig. 6. Growth showing the effect of the worms on it.

Fig. 7. Growth on pebbles used as control, at commencement of experiment.

Fig. 8. Growth on pebbles before introduction of the worms.

(*Received* 13 *September* 1938)



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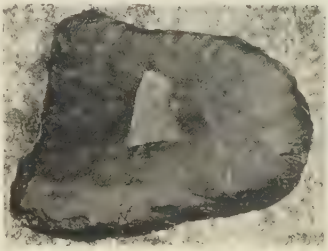

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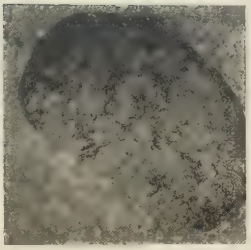

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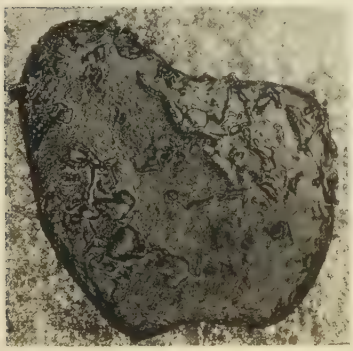

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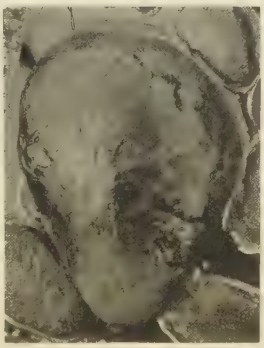

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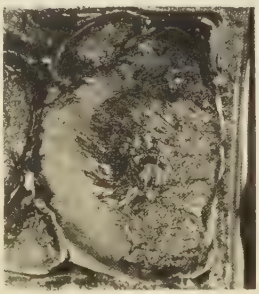

Fig. 6.

Fig. 7.

Fig. 8.

REYNOLDSON.—Enchytraeid Worms and the Bacteria Bed Method of Sewage Treatment
(pp. 138–164)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ORDINARY MEETING of the Association of Applied Biologists held in the Imperial College of Science and Technology, London, on Friday, 7 October 1938. The morning session began at 11.45 a.m. in the Botany Lecture Theatre, the Chair being occupied by the President, Mr C. T. GIMINGHAM. The afternoon session began at 2.30 in the Botany Lecture Theatre, the Chair being taken by a Vice-President, Dr H. MARTIN.

Discussion on Fresh-water Biology and its Applications

The following papers were read:

I. Fresh-water biology and its applications: Introduction. By E. B. WORTHINGTON, M.A., Ph.D.

II. Physical and chemical aspects of organic production in lakes. By C. H. MORTIMER, B.Sc., Dr.Phil.

III. Algal physiology and organic production. By MARIE ROSENBERG, Dr.Phil.

IV. Some aspects of waterworks biology. By A. C. GARDINER, M.A.

I. FRESH-WATER BIOLOGY AND ITS APPLICATIONS: INTRODUCTION

BY E. B. WORTHINGTON, M.A., PH.D.

Director of the Freshwater Biological Association, Wray Castle, Ambleside, Westmorland

FRESH-WATER biology is of direct use to mankind in three different ways, in connexion with water supply, with fisheries, and with teaching. The last can be left out of account for present purposes, and of the other two it seems best to devote most attention in this discussion to water supply. The object of this introduction is to formulate the general problems, leaving others to give facts and take up specific questions.

In water supply and fisheries, just as in agriculture, the major biological problems are concerned with production of life, but research on the productivity of waters is still far behind that of the land. Between the two aspects of water supply and fisheries there is a big distinction—that water supply in general requires a reduction of productivity in order to avoid algal and other obnoxious growths, whereas fisheries, like agriculture, require an increase of productivity throughout all stages in the food cycle. The extent of the increase in productivity required is, however, dependent on the species of fish, because different fish, like crop plants, vary in their ecological requirements. The distinction in object mentioned above is not so great as it sounds, because

in order to increase or reduce productivity, we must know the factors by which it is controlled in nature. Moreover, the two aspects of reduction and increase may have to be considered together, because many natural waters carrying stocks of fish are used for supplying water, and many artificial reservoirs constructed for the storage of water are used for fishery purposes.

On the fishery side it is possible to make comparisons between the production of fish and of domestic animals or crops in terms of weight per unit area (see Dr Mortimer's paper). The conclusion is that, whereas production from fresh-water cannot compete with that from good agricultural land, it may do so with some types of poor forest or grazing land, so that under certain circumstances it may actually pay to convert land into water. Here there are two important considerations, (1) that land which can be converted into water most easily is usually waterlogged and of little use agriculturally, and (2) the sporting aspect of fresh-water fisheries is important: for example, the economic value of a trout lake depends on the amount of money which changes hands as a result of angling, and this sum is usually several times the market value of the fish caught. There may be opportunities in the future for increasing the production of water in this country up to the optimum for different kinds of fish, by manuring, a branch of biology which has been applied with great success on the continent in relation to the carp. The salmon, which provides the most valuable of Britain's fresh-water fisheries, from both netting and sporting points of view, presents many special problems which fall outside the scope of this discussion.

On the side of water supply, applications of biology are wanted mainly in connexion with the storage of surface waters rather than the derivation of underground supplies. The field for future work has been cleared to some extent by a short article by Prof. W. H. Pearsall in the *Annual Report of the Freshwater Biological Association for* 1936, and some of his conclusions may be quoted. The underlying principle is that results obtained from the study of natural waters, particularly of lakes, are applicable with modifications to reservoirs. Every environment is subject to change in its natural conditions, and in water this change is in general towards greater production of life. A lake or a river in a given set of geological and climatic conditions is subject to erosive forces. Sediment accumulates from the earliest days in the case of lakes, in the later stages of erosion in the case of rivers, and this sediment alters the chemical content of the water, and in shallow areas gives a hold for rooted vegetation. The accumulation and decay of dead animals and plants alters the composition of the deposits, the general tendency being towards an increase of organic matter. Thus any group of lakes or rivers can be arranged in a series which represents in broad outline the stages through which each will pass during its evolutionary history. It has been shown that in shallow water the larger plants, littoral algae, and bottom fauna vary in kind and number with these stages of development of bottom deposits. The same influences affect the free-swimming organisms, particularly the fish and insects. In a deep lake or reservoir, where the littoral zone is unimportant, the production of life centres round the plankton in its relation to the water and deep deposits. As the deposits increase in organic matter, so also do the nitrogen and phosphorus reserves increase in the mud; these are capable of being delivered into the water, especially at times of annual turn-over, so that evolved lakes or reservoirs may become increasingly independent in regard to chemical substances, and no longer influenced to so large an extent by the character of the inflowing water.

Now, regarding the application of these principles to reservoirs, the accumulation of nitrogen and phosphorus is highly undesirable because it involves increased productivity of algae. The rate at which their accumulation progresses depends on the situation: thus some reservoirs which were formerly pure water lakes, such as Thirlmere, progress very slowly, and indeed have changed but little in the past 15,000 years or so since the ice age. But where land has been converted into water, as in many reservoirs in England, progress may be very much more rapid: there are in fact cases where production has increased greatly during 50 or 60 years in the north of England, while in the south of England the rate of silting and increased production may be still higher, so that reservoirs constructed at the beginning of this century are already giving serious trouble.

Obviously some means must be developed of maintaining the *status quo* in reservoirs, and Pearsall suggests three possibilities: (1) the removal of nitrogen, phosphorus, etc., from inflowing waters, (2) the removal of mud from the bottom, which is possible in the case of series of reservoirs, where one or more can be put out of supply, (3) annual cropping to remove an amount of organic matter equivalent to the annual gain. It is clear that in many cases it should be easier and more efficient to remove the crop in the form of fish rather than plants, plankton, mud or bacteria. Hence, the conclusion is reached that an increased crop of fish may actually reduce the likelihood of trouble from the smaller forms of life which clog filter beds or otherwise lead to difficulties in the provision of pure water.

The above paragraphs outline the fundamental problem in the application of fresh-water biology to water supply, but before it can be solved, it is necessary to answer, albeit approximately, such questions as—in a given lake, reservoir or river what is the total stock of living plants and animals, and, in order to avoid an increase in the stock, what is the annual crop which must be removed? Answers to such questions would solve many problems with which the water undertaker is confronted to-day. They would help him in the siting of new reservoirs, in estimating the extent of filter beds required, in controlling inflowing waters of different chemical content in order to keep algae at a minimum, in deciding from what levels to draw water at different times of day and during the year, and how far it is desirable to stock reservoirs with fish and to encourage angling. In some of these questions knowledge is now sufficient to be of direct value, as the subsequent papers will show.

II. PHYSICAL AND CHEMICAL ASPECTS OF ORGANIC PRODUCTION IN LAKES

By C. H. Mortimer, B.Sc., Dr.Phil.

Assistant Naturalist, Wray Castle, Ambleside, Westmorland

Introduction and definitions

For the purposes of this paper the *productivity* of a lake is defined as the organic matter produced by the planktonic algae (phytoplankton). Production by rooted shore plants may be neglected in a large body of water. Production in this narrow sense is distinct from that in which it is frequently employed, namely, the production of all forms of life including the heterotrophic animals and bacteria. Heterotrophic forms merely consume organic matter produced by plants.

The *potential productivity* is determined by physical and chemical factors. Of the former light and temperature are important; they will be discussed in more detail in the following paper, but for present purposes it may be assumed that in most lakes little photosynthesis takes place below 15 to 20 m. Of the inorganic elements necessary for plant growth nitrogen and phosphorus are normally only present in small quantities in the water, so the depletion of one or other of these *limiting substances* may hold up production. Potential production is therefore directly determined by the rate of supply of the substances in question. Seasonal changes in light and temperature impress a seasonal rhythm on the organic cycle in a lake. In winter, lake water is more or less equal in temperature from top to bottom, and currents produced by the wind keep it in complete circulation. This results in the distribution throughout all depths of dissolved oxygen taken in at the surface, and of the nutrient salts produced by bacterial decomposition of organic matter in the mud. During the summer the surface waters warm up and become less dense. The strength of the wind-produced currents is not sufficient to force this warm, light water down into the denser cold water, so the circulation becomes confined to an upper layer, the *epilimnion*. This lies above a lower, colder stagnating layer, the *hypolimnion*, and there is a zone of sharp temperature change between them, the *thermocline*. In most cases the thermocline is at such a depth (15–20 m.) that photosynthesis is practically confined to the epilimnion. The algae produced here eventually sink into the hypolimnion and decompose there, or in the mud with which it is in contact. Organic elements are thus removed from the upper producing layers during the summer, and only return into circulation again when the lake cools down and "turns over" in the winter. Organic production is, therefore, controlled by the amount of the limiting substances present in the epilimnion at the beginning of such a stagnation period.

The seasonal cycle in a natural lake

The cycle in Windermere may be said to start with diatom growth in the spring, and it reaches its maximum at the beginning of June (Fig. 1). This growth is checked (Fig. 2) by the gradual, and eventually complete depletion of one or more nutrient salts in the epilimnion, which by this time is beginning to form. Conditions in the epilimnion after such a diatom maximum are characterized by constant depletion of minimum substances and an increased content of dissolved organic matter. These conditions are favourable to, and support, a population of blue-green algae, which, however, remain at a far lower level of production than that attained by the diatoms. Only a part of the organic capital produced by the diatoms in the spring is utilized by succeeding forms, such as the zooplankton (Fig. 1), in the same year. A large part of the crop falls into the hypolimnion, out of circulation, for that year. *Asterionella* contains approximately 50% dry weight of silica. The amount of diatom production calculated from the silicate depletion curve (Fig. 2) is in excess of the diatom dry weight actually found in the surface water.

Decomposition, with resulting mineralization in the hypolimnion water, and in the mud, uses up a part or all of the oxygen layer and enriches the hypolimnion with nutrient salts which, however, do not become available to the upper photosynthesizing layers until the end of the stagnation period. This cycle of events—i.e. production, followed by consumption and mineralization—with the seasonal rhythm imposed by thermal stratification, is similar in many, but not all, lakes and reservoirs (see

Mr Gardiner's paper). Phosphate is more often a limiting substance than nitrate. Silicate may limit diatom production (Fig. 2). In all cases the potential production is controlled by the rate of supply of the limiting substances from the mud and from the drainage basin. As the rate of supply from the mud depends on the original supply from the drainage basin it is clear that geological and climatic factors ultimately control organic production in water. In waters receiving relatively large concentrations of

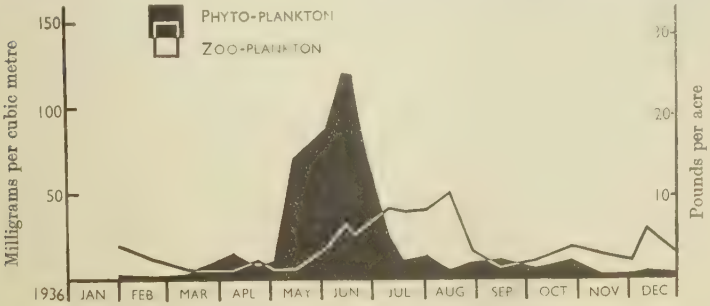

Fig. 1. Plankton production (dry weight) in Windermere.[1]

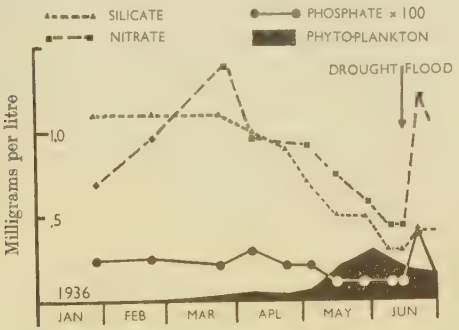

Fig. 2. Phytoplankton growth (dry weight) and nutrient salt depletion in Windermere surface water.

limiting substances (nitrogen and phosphorus) in the form of sewage effluents or drainage from agricultural soil, production is obviously increased. The influence of bases (calcium and magnesium) on organic production is also important. High organic production is rarely found in non-calcareous waters. The importance of climatic factors is seen in the effect of a flood after a drought in increasing the nutrient salt concentration (Fig. 2). Pearsall has shown that heavy floods in the early months of the year were followed by high spring diatom crops in Windermere. The converse was also true. It would appear that substances washed into the lake by these floods had increased potential production.

[1] Assuming same distribution throughout lake.

The measurement of production

For the study and scientific control of production some method of measurement is essential which will give results as crop weights per unit volume or area. The following are some methods which have been applied:

(1) Periodic sampling of the various forms of organic matter in the water can supply data for the calculation of the standing crop. For approximate measurements, and these are as much as one can usually hope to obtain, the zooplankton may be regarded as being the sample retained by a net of coarse silk (60 meshes to the inch), and the phytoplankton as being that which is retained by the finest net (180 meshes to the inch). This has been done in Fig. 1.

(2) Colorimetric or spectrographic estimation of the plant pigments, chlorophyll and others, extracted with various solvents, enable the estimation of plant material in a mixed sample to be made.

The above methods give a more or less accurate estimation of the standing crop at any one time. Until the algal physiologist can supply data of the reproduction and death rates of the various organisms under different conditions, the *gross production* of total weight or total numbers of organisms in one season can only be estimated indirectly by one of the following methods:

(3) An approximate assessment from standing crop figures on the estimation of fifty changes in stock per annum has been made for Lake Mendota. The gross production in one season was 10,700 lb./acre dry weight. The amount of zooplankton or fish allows one to gauge the general level of production. These animals have longer life cycles than the phytoplankton and more or less smooth over the large seasonal fluctuations which planktonic algae show.

(4) In special cases the extent and rate of depletion of a minimum substance enable the rate and extent of gross production to be measured with some accuracy. It is sometimes possible to prophesy when a certain substance will become depleted and stop growth. A case in point is the silicate depletion by diatoms in Fig. 2.

(5) It has been suggested by Strøm that the oxygen deficit arising in the hypolimnion at the end of a stagnation period is proportional to the amount of organic matter falling from the epilimnion and, therefore, to gross production during that period. If this deficit is corrected for reducing substances which appear if the oxygen is completely used up, and calculated per unit area of hypolimnion surface, the ratio of this figure to the main standing crop during the stagnation period is constant in lakes of widely differing productivity (Table I).

Table I[1]

Lake	Green Lake, Wisconsin	Lake Mendota, Wisconsin	Furesö, Denmark	Black Oak Lake, Wisconsin
Mean standing plankton crop in kg. dry matter/hectare	277	240	157	94
Ratio	100	89	62	36
Real oxygen deficit since turn-over in mg./cm.^{2} of hypolimnion surface	12·82	11·39	7·92	4·57
Ratio	100	87	57	34

[1] Compiled from data from Hutchinson, G. E., *Internat. Rev. Hydrobiol.* (1938), **36**.

Limitations of the present data, and possibilities of production control

The quantitative data of production biology outlined above are extremely meagre, but it is the lack of qualitative data which constitutes the most serious gap in our knowledge. Chemical work both in fresh waters and in the sea has clearly shown the effect of the depletion of minimum substances in limiting production, but it has little

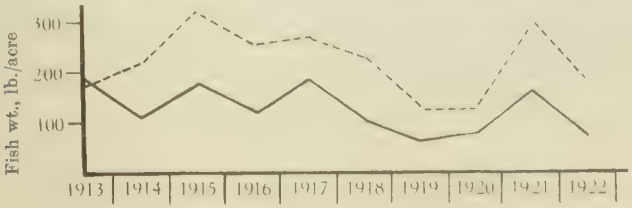

Fig. 3. Annual crop production in manured and unmanured carp ponds.[1]
---- Manured with phosphorus and potassium. —— Unmanured controls.

to say about what starts the growth of an algal population. We are far from the ideal of being able to prophesy from a water analysis which algae will grow, and under what conditions they will appear. Cases in which all the minimum substances usually tested for in analyses are abundant, and no algal growth occurs, are not uncommon, whereas

Table II. *Annual crop weights in farming and fresh-water fisheries compared*

Type of farming	Food produced lb./acre/annum	Type of fishery	Food produced lb./acre/annum
I. Grazing:		Windermere*	Approx. 1·5
Meat the only product:		Alpine lakes†	Approx. 6·4
(*a*) From rich pasture	190	(average 10 lakes)	
(*b*) From poor pasture	20	*Carp ponds*‡	
		Unmanured	100
II. Dairy farming:		Manured	190
Meat and milk joint products:			
(*a*) Milk	2,000		
(*b*) Meat	45		
Total produce per acre	2,045		
III. Mixed arable farming:			
Various arable crops grown:			
(*a*) Wheat	1,950		
(*b*) Potatoes	17,920		
(*c*) Sugar beet	3,043 as sugar		

* Allen, K. R. Personal communication.
† Haempel, O. *Die Binnegewässer* (1930), **10**.
‡ Demoll, R. Teichdüngung. *Handb. Binnenfischerei Mitteleuropas* (1925), **4**.

in an apparently identical body of water a large outburst of growth may appear. It seems likely that there are yet more minimum substances stimulating algal growth of which we know nothing. The effect of winter floods on the Windermere diatom maxima, the productiveness of soil drainage in general, and the success of soil extract for

[1] Data from Demoll, R. Teichdüngung. *Handb. Binnenfischerei Mitteleuropas* (1925), **4**.

culturing all sorts of organisms, suggest that such unknown growth substances may be present in the soil. Although the lack of such essential data seriously limits the application of present knowledge to every case where the control of production is of practical importance, some attempts at chemical control have been very successful. Such a case is shown in Fig. 3. The addition of the limiting substances, phosphorus and potassium, increased the fish crop in all years above that of the controls, and the increase was proportionately greater in good years. A comparison of the crops produced in such artificial carp ponds, in natural lakes, and on agricultural land is of interest (Table II). Although meat production in most natural fisheries falls far below that produced in agriculture, intensive fish production on waste land, utilizing such waste products as sewage sludges and effluents might be an economic possibility.

III. ALGAL PHYSIOLOGY AND ORGANIC PRODUCTION

By Marie Rosenberg, Dr.Phil.

Assistant Naturalist, Wray Castle, Ambleside, Westmorland

In any problem connected with the balance of life or the production of organisms in water, the algae play a very important part. The general term "algae" covers a vast number of species of lower plants found in habitats differing widely in combinations of the factors important to plant life, namely light, temperature, and nutritive substances. Thus, some species live typically on melting snow at the edge of glaciers, others in hot springs; well characterized algal communities are known from waters with no detectable dissolved substances at one extreme, whereas practically saturated solutions of sodium chloride may show abundant development of Volvocales. A great variety of possible and actual combinations lies between these extremes in habitats, including many types of so-called fresh waters, with which we are concerned here.

These few examples give some idea of the range of habitats from which problems of ecology or physiology may be selected, all leading up to general questions concerning productivity.

We can distinguish, roughly speaking, three groups of processes going on in the water just as on land—production connected with the autotrophic plants, mainly the phytoplankton composed of algae, consumption connected with the animals, and reduction due to the work of bacteria. Chemical processes in the water have to be the principal basis for our understanding of the distribution of algae in space and in time. Comparative work in various countries has shown that it is possible to distinguish different types of waters, and to classify them as transition stages in an evolutionary series. In this connexion detailed work on a number of lakes has given interesting information as to the requirements of different groups of algae, and of their succession. Conclusions drawn from such observations must, however, be tentative, and must be checked by experiment. This second stage of causal analysis has not progressed very far, but it is of great importance to the theoretical and practical problems involved.

An annual periodicity in the numbers and species of phytoplankton has long been observed, maxima in spring and summer being the most striking general phenomena. The three groups of factors, light, temperature, and dissolved, nutritive substances, are being studied at the same time and correlated with the biological results. Production

of phytoplankton can only commence if and when the necessary conditions for cell division are fulfilled. The rate at which every single cell divides under a given set of conditions will be of utmost importance for the whole balance of chemicals and the supply of organic food for the heterotrophic organisms. Obviously, therefore, the conditions must be studied under which any given species or group of species attains its optimum rate of division.

The first way of approach, field work, has been followed for many years, with ever improving technique in all branches concerned. For example, the penetration of light into water is now measured with photoelectric cells calibrated in physical units, thus expressing light in absolute values. This has been done in many cases and the results show interesting variations for different waters of the extension of the zone in which photosynthesis can take place. Thus, in some of the English lakes the same light intensity has been found in Bassenthwaite at a depth of 4 m., Windermere at 8 m., and Ennerdale at 21 m., readings having been taken in similar conditions. These figures, in conjunction with other data, express the productivity of the three lakes. Bassenthwaite was having at the time a huge diatom maximum, Windermere showed lower figures of plankton organisms, and the plankton of Ennerdale was very poor indeed. This example shows well the interrelationship of various factors. Although plenty of light was available in Ennerdale practically no plankton production was possible owing to a deficiency in dissolved substances. In Bassenthwaite, on the other hand, the mass production of plankton, developed owing to abundant dissolved substances, functioned as a screen and cut down the light for the underlying layers, thus limiting further production.

Considering temperature, the general principle holds that production increases with increasing temperature. Although, in some cases, optimum temperatures for the production of algae have been given, it is by no means clear that temperature is a factor of special importance for the distribution of phytoplankton. The importance of temperature for the distribution of chemicals, however, is clear, and certainly in this secondary way temperature changes are of great importance for the phytoplankton. This leads to the third and very important group of factors, the dissolved substances in the water.

In Dr Mortimer's paper the annual variation of several biologically important substances has been shown. As a demonstration of their importance for the algal flora a short outline of the periodicity of algae in a lake such as Windermere may be given. The general trend is a diatom maximum in the spring, followed by blue-green algae, then colonial green algae, Desmids, with another smaller diatom maximum in the autumn. The winter shows very low figures for all groups concerned, most forms disappearing from the plankton. The chemical changes in the water throughout the year justify the following conclusions. Diatoms, especially *Asterionella*, occur as dominants when the water is richest in nitrates, phosphates and silica. Volvocales and Desmids occur in largest numbers when nitrates and phosphates are low. Desmids particularly need a low calcium and nitrate phosphate ratio for their maxima. Blue-green algae show their maximal growth when the organic matter dissolved in the water is high; they can grow rapidly in small quantities of nitrates and phosphates. These conclusions have been confirmed by comparative studies of several of the English lakes. Although geologically similar, the drainage areas differ mainly in their amount of agricultural land, thus supplying the lakes with very different amounts of bio-

logically important dissolved substances and silt. The lakes in question can be understood as successive stages of development from a poor or primitive to a rich and highly productive type. The main and first indicator for this change is the type of phytoplankton showing the same correlation with dissolved substances, as has been shown for the annual cycle in an individual lake. Tables I and II, taken from Pearsall, illustrate this:

Table I

Lake	Desmids	Green Colonial	*Dinobryon*	Desmids Colonial green	N/P
Ennerdale	76·0	17·0	—	4·5	9·6
Crummock	11·6	5·5	31·0	2·1	13·5
Wastwater	39·0	23·0	11·0	1·7	24·0
Derwentwater	7·5	3·5	39·0	2·1	13·8
Bassenthwaite	13·0	1·5	1·5	8·6	53·0
Lowes Water	10·5	1·5	4·0	7·0	49·1
Windermere	7·0	12·0	10·0	0·6	60·6
Ullswater	2·5	0·5	0·5	5·0	33·6
Esthwaite	2·0	5·5	1·5	0·36	54·5

Table II

	Albuminoid NH_3 in June	% of Myxophyceae in July
Bassenthwaite	0·010	0
Wastwater	0·026	0
Derwentwater	0·035	3
Ennerdale	0·038	7
Ullswater	0·045	2
Crummockwater	0·057	19
Windermere	0·061	26
Esthwaite	0·070	27
Windermere	0·075	46
Bassenthwaite	0·080 (July)	51 (August)
Lowes Water	0·082	61

Most of the examples mentioned imply the conception of a limiting factor of some kind. As in most other branches of biology it has become increasingly clear that it is impossible to consider one isolated factor alone. In fresh-water biology, especially in the case of the phytoplankton, it has become quite clear that we are always dealing with a complicated combination of factors and that the relative values of these may differ immensely in new combinations. We are always dealing with a certain *set* of conditions as a whole and must be careful to keep this in mind. One chemical substance, for instance, may have a most striking effect on division rate when present with another, and may fail entirely or even have an unfavourable or toxic effect when present in a different combination. The last word in all these cases must necessarily be left to the experiment, for this can clear up details otherwise inaccessible if field work alone is considered. Comparative experimental work on cultures under standard conditions has shown the usefulness of such controlled work, and it is hoped that the knowledge of the physiology of plankton algae will be furthered greatly by systematic experimental work—always of course in touch with conditions in nature.

IV. SOME ASPECTS OF WATERWORKS BIOLOGY

By A. C. Gardiner, M.A.

Biologist, Metropolitan Water Board, London

Dr Mortimer has discussed the question of productivity in lakes and has used, in the main, data derived from Windermere. It is my task to show how this information can be applied to the reservoirs of the Metropolitan Water Board, which may be regarded as small lakes, receiving a river water rich in nutrient salts. In London, at any rate, the algal flora of the reservoirs is markedly different, both qualitatively and quantitatively, from that of the raw rivers from which they are supplied. As a rule, a very rich plankton develops seasonally in the reservoir. Concentrations of over 3 million individuals per litre of the diatom *Stephanodiscus astraea* have been recorded and even larger numbers of other forms. What this means in practice may be more readily appreciated if I say that in a large reservoir, holding some 6750 million gallons, the standing crop of the diatom *Fragilaria crotonensis* had, at the time of the spring maximum, an estimated dry weight of 110 tons. Although water was being drawn from near the bottom, where concentration of algae was less, it meant that the dry weight of *F. crotonensis* which had to be filtered off from this water was in the region of a ton a day.

In the case of the larger reservoirs it is now possible to forecast the probable duration of an algal outbreak. The method underlying such forecasting is based upon a knowledge of the factors controlling algal production. The date on which a particular growth is more likely than not to come to an end is arrived at from a study of the rate of algal increase and the rate of consumption of certain essential salts. The former is preferably obtained by counting, the latter by chemical analyses at intervals of not more than one week. The accuracy of the forecast depends, to some extent, upon stable weather conditions and necessitates a study of the salt content of the raw river water feeding the reservoir. The two dissolved substances, which have so far proved most useful in this respect, are phosphates and silicates. Briefly, if the rate of natural increase of the algae is such that the amount of phosphate and silicate is depleted more rapidly than fresh supplies are being brought in from the river, the time will come when concentrations of these substances fall to very low levels. When this occurs, the division rate slows up and finally the algae disappear from the plankton. The practical value of such a forecast lies in the fact that it enables one to decide whether remedial measures should be attempted or whether an intensive period of filter-bed cleaning will not suffice.

This method of forecasting is not possible in all cases. Where the ratio of volume of incoming water to volume impounded is high, the water in the reservoir is changing so rapidly that there is little chance of the salt-utilization rate of an algal population gaining on the rate of replenishment from the river. In other words, a high concentration of algae can be maintained with no marked fall in the salt content. What brings such an algal maximum to an end I have no idea. In the case of the diatoms, it may be that a relatively prolonged period of multiplication reduces the size of the resulting frustules to the minimum for the species, but for other classes of algae this does not apply. Save in a few cases, one hesitates to invoke temperature as a factor

of much moment: about the influence of light intensity our information is scanty, but I should doubt if it were decisive.

In such cases, remedial measures must be put in hand to deal with excessive growths. The two methods most commonly employed are the use of algicides, such as copper sulphate, or the application of a coagulant, like alum or ferric hydroxide. Neither is a sure remedy. The use of copper sulphate is attended by the risk of after-growths in the form of copper-resistant unicellular Chlorophyceae. Whilst alum has been found very successful in some cases, notably those of the diatoms *Stephanodiscus Hantzschii* and *St. astraea*, it has not proved of much use for *Fragilaria crotonensis*.

The last point in connexion with reservoirs is the difference between those in normal use and those which are allowed to stand. Any water undertaking which relies upon a number of relatively small storage basins must maintain a reserve above normal requirements, which reserves can be used in times of drought. This means that some reservoirs may be allowed to stand for a period of time which, if there be no drought, may be up to several years.

The most striking difference between the chemistry of a reservoir in normal use and one which has been allowed to stand is, perhaps, the alteration in the ammonia-nitrate ratio. In reservoirs in normal use, concentrations of nitrate are in the region of 0·17–0·22 part N/100,000, whilst those of ammonia are 0·002–0·007 part N/100,000. This relatively high concentration of nitrate is of some importance, because if we make the assumption that nitrogen as nitrate is the form most readily assimilated by diatoms, then it can be shown, from the ratio of nitrogen to phosphorus in the plant body, that nitrate can never be a limiting factor. In a standing reservoir, on the other hand, not only does the concentration of nitrate fall seasonally below that of ammonia, which for its part rises, but may become so low as now to be a limiting factor. In one reservoir, which has stood for a long time, concentration of nitrate this year has never exceeded 0·05 with a mean (January to October) of 0·009 part N/100,000. On many occasions the amount present was too low to be measured by the reduction method used. Since concentrations of phosphate and silicate rise when a reservoir is put out of normal use, the drop in nitrate, which I believe must result very largely from the activity of nitrate-reducing bacteria, assumes considerable importance. Had it remained normal, a really enormous algal production would have seemed inevitable.

Three cases have recently been investigated of reservoirs which have stood idle for some years. All are peculiar in showing a very heavy growth of the blue-green alga, *Oscillatoria rubescens*, during the colder months of the year. I am unable to say whether this growth is the direct result of the low nitrate content, which in turn, I believe to be correlated with standing idle; I can only say that it looks as if Cyanophyceae can flourish at very much lower levels of nitrate concentration than can diatoms. This is interesting, since analyses have shown that the percentage of nitrogen, expressed on an ash-free basis, is higher in planktonic Cyanophyceae than in diatoms. A consideration of the conditions prevailing during the prolonged period when *Osc. rubescens* was abundant in one of these standing reservoirs has led me very tentatively to suggest that this, and probably other blue-green algae, can fix atmospheric nitrogen. There is some evidence from the literature that certain Cyanophyceae can do this, but it is desired to emphasize that my own observations do not, as yet, entitle me to express any definite opinion.

The possibility that there is a direct connexion between concentration of nitrate

and production of Cyanophyceae raises a question of practical importance. Although *Oscillatoria* is readily removed by filtration, the disposal of sand wash-water presents considerable difficulties, since, on standing, it develops a bad smell and may be of an intense and lurid colour. To avoid a large production of the species, therefore, it might be advisable to advocate pumping river water into such reservoirs in order to maintain the nitrate concentration above that at which there is the risk of the appearance of *Osc. rubescens.*

Finally, I should like to draw attention to a subject which seems to me of some importance. The applied biologist is commonly called upon to deal with problems for which he has neither the answer nor the time in which to look for it. Some of these problems are capable of being tackled in Universities or in Research Institutions. It would undoubtedly be to our advantage if those in charge of what may be termed the more academic research laboratories could make a selection from such problems—many quite fundamental—which the applied biologist is expected to answer, as often as not on the telephone.

THE ASSOCIATION OF APPLIED BIOLOGISTS AND THE *ANNALS OF APPLIED BIOLOGY*— A RETROSPECT (1904–38)

By WILLIAM B. BRIERLEY

University of Reading

(With Plates X–XIII)

With the close of 1938 the *Annals of Applied Biology* completed its first 25 volumes, and the occasion appeared to be a suitable one on which to review progress in the Association and its journal.

I am indebted to various senior Members who read this account in typescript and gave me the benefit of their greater knowledge and experience.

I. The Association of Applied Biologists

Prior to the year 1904 there was, in the United Kingdom, no scientific society or scientific journal devoted to applied biology. The active workers in the field were comparatively few, but the need was becoming evident for some central exchange and clearing house of ideas and research. In 1904, Mr Walter E. Collinge of Birmingham University raised the question of the founding of a society to serve these purposes and to advance the study of the applied aspects of biology. The idea was canvassed privately among a few interested people, and on the initiative of Mr W. E. Collinge and Mr F. V. Theobald, a meeting of workers interested in applied biology was called in the rooms of the Linnean Society of London on 8 November 1904.

There were present, Prof. G. S. Boulger, Mr W. E. Collinge, Dr H. T. Güssow, Mr E. M. Holmes, Mr A. E. Shipley and Mr Herbert Stone, with Mr F. V. Theobald in the Chair. "In a few introductory remarks the Chairman detailed the steps taken by Mr Collinge to found an Association of Economic Biologists. He hoped that it would welcome all investigators in Economic Biology, whether Agricultural, Medical, or Commercial. The interdependence of Biology, Agriculture, Medicine, and Commerce was apparent to all."

The Association of Economic Biologists was then inaugurated with the following Original Members: Prof. G. S. Boulger, Prof. A. H. R. Buller, Prof. G. H. Carpenter, Mr J. B. Carruthers, Mr W. E. Collinge, Prof. W. R. Fisher, Mr A. T. Gillanders, Mr E. M. Holmes, Mr D. Houston, Mr A. Howard, Mr A. D. Imms, Mr E. J. Lewis, Dr A. Stewart MacDougall, Dr F. H. A. Marshall, Mr H. Maxwell Lefroy, Mr Robert Newstead, Dr G. H. Pethybridge, Prof. Ronald Ross, Mr A. E. Shipley, Dr W. Somerville, Mr Herbert Stone, Mr Fraser Story, Mr F. V. Theobald, Mr R. Hedger Wallace, Mr Cecil Warburton, Mr F. C. Wilcocks.

Mr Collinge and Mr Theobald had drafted a Provisional Constitution and Laws, and this, with slight emendations, was approved and ordered to be printed.

The following were nominated for Office for 1905: President—Mr F. V. Theobald; Vice-Presidents—Mr A. E. Shipley, Dr William Somerville; Hon. Treasurer—Mr Herbert Stone; Hon. Secretary—Mr W. E. Collinge; Council—Prof. G. S. Boulger, Prof. A. H. R. Buller, Prof. G. H. Carpenter, Dr F. H. A. Marshall, Mr Robert Newstead, Prof. Ronald Ross, Mr Fraser Story, Mr Cecil Warburton; Publication Committee—Mr E. M. Holmes, Mr A. E. Shipley.

It was decided to print and distribute among biologists a leaflet setting forth the objects of the Association. This stated: "The objects of the Association are to discuss new discoveries, to exchange experiences and carefully to consider the best methods of work. To give opportunity to individual workers of announcing proposed investigations, so as to bring out suggestions and prevent unnecessary duplication of work. To suggest, when possible, certain lines of investigation upon subjects of general interest, and generally to promote and advance the science of Economic Biology in its agricultural, horticultural, medical, and commercial aspects. The work of the Association will include the various problems connected with economic botany, such as the fungoid diseases of plants and animals; those connected with economic zoology, such as the many problems in connexion with insects and other animals injurious to crops, live stock, animal parasites, etc., the scientific cultivation of plants and breeding of animals, and the questions affecting the various natural history products that enter into commerce." "Membership shall be confined to workers in Economic Biology. All such Biologists employed by the Government or by any County or City Council, University, or Agricultural or Horticultural College or Association, and all persons engaged in investigations in Economic Biology may become Members." "Persons engaged in practical work in Economic Biology (Fruit Growers, Breeders of Live Stock, etc.) may be elected as Associates." There were also to be not more than ten (later increased to twelve) Honorary Members who "shall be persons (not subjects of the British Crown) who have contributed in an eminent degree to the advancement of the science of Economic Biology". Not more than four meetings were to be held each year.

The second meeting of the Association (the first scientific meeting) was held in the University of Birmingham on 19 and 20 April 1905, when eleven scientific papers were contributed. A dinner took place on 19 April at 7 p.m. in the Acorn Hotel, Birmingham, followed by a "Smoke and Chat" in the Zoological Department of the University. The meeting was attended by twenty-five Members and seventeen visitors, and twenty-three new Members and seven Associates were elected. The following were elected Honorary Members: Prof. A. Berlese, Prof. R. Blanchard, Prof. N. A. Cholodkovsky, Dr A. D. Hopkins, Dr L. O. Howard, Dr A. Looss, Prof. G. L. Neumann, Prof. J. Ritzema-Bos. A pamphlet containing abstracts of the papers read to the meeting, together with the text of a paper entitled "A plea for the study of British Aphides in connexion with cultivated plants", by Mr F. V. Theobald, was issued as *Proceedings of the Association of Economic Biologists*.

The third meeting (second scientific meeting, and first annual general meeting) was convened in the Liverpool School of Tropical Medicine on 28 and 29 December 1905. In presenting the first Annual Report the "Council have to congratulate the Members of the Association on the position and numerical strength attained in the short time

which has elapsed since the foundation of the Association. The Inaugural Meeting was held on 8 November 1904, with twenty-seven Original Members,[1] and the number of Members on 21 December 1905 stood as follows:

Honorary Members	8
Ordinary Members	50
Associate Members	10
Total	68"

It was also noted in the Annual Report of the Council that "The total receipts up to December 25th were £33. 8*s*. 6*d*., including one life subscription of £7. 7*s*. 0*d*.; whilst the total expenditure for the same period amounts to £28. 17*s*. 11*d*., leaving a balance in the hands of the Honorary Treasurer of £4. 10*s*. 7*d*." Thirty Members and visitors attended the meeting and sixteen new Members and two Associates were elected. The President delivered the first Presidential Address on "Animal parasites and legislation", and nine other papers were contributed.

No meeting was arranged during 1906 but, in 1907, two meetings were held. The first of these took place in the Pathological Department of the University of Cambridge on 9, 10 and 11 January, and amongst the fourteen communications, one by Mr R. H. Biffen dealt with "Cereal breeding", and another by Mr E. S. Salmon was entitled "On the American gooseberry-mildew; an epidemic fungus disease now invading Europe". A second meeting was convened in the Imperial Institute, London, on 4 July 1907. Among the papers read was one by Mr A. D. Imms on "A disease of bees in the Isle of Wight", and one by Mr E. S. Salmon on "The American gooseberry-mildew, and the proposed legislative measures".

During 1908 two meetings were held, the annual general meeting at University College, London, on 15 April, and an Ordinary Meeting at the University of Edinburgh on 28, 29 and 30 July. A paper contributed to the London meeting by Mr C. Gordon Hewitt was entitled "The need of an organized enquiry into the feeding habits of British birds", and led to the moving of the following resolution: "That in the opinion of this Association it is desirable that a committee should be formed for the investigation of the feeding habits of British birds; the results of the work of such an Economic Ornithological Committee would be of very great importance in that it would obtain precise information concerning the economic habits of these birds." Such a committee later came into being and advanced in notable degree our knowledge of this problem. The Edinburgh meeting was rather sparsely attended but, among the fourteen papers contributed, were "Rats and their animal parasites", being the Presidential Address by Mr A. E. Shipley; "The action of Yohimbine on the generative organs", by Dr W. Cramer and Dr F. H. A. Marshall; and "Inbreeding and other experiments", by Prof. Cossar Ewart.

In 1909 an annual general meeting only was held, on 13, 14 and 15 July, in the School of Forestry of Oxford University. Among the papers read to the meeting were one by the President, Dr A. E. Shipley, dealing with grouse disease and entitled "The relation of certain Cestode and Nematode parasites to bacterial disease"; "The actual and possible application of recent discoveries in heredity to economic problems", by Mr A. D. Darbishire; "A successful curative treatment of Piroplasmosis", by Prof. G. H. F. Nuttall and Dr S. Hadwen; and "Investigations of the large larch sawfly,

[1] The names of Original Members listed in the minute book number 26.

Nematus erichsoni", by Dr C. Gordon Hewitt. In the fourth Annual Report of the Council presented at this meeting it was noted that the total number of Members of all classes on 30 June 1909 was 132, and that the balance in the hands of the Hon. Treasurer amounted to £86. 19*s.* 1*d.*

During the years 1906–9, four annual parts of the *Proceedings of the Association* had been issued. With the appearance of the fourth part in July 1909, the *Proceedings* were discontinued, since arrangements had been made for Members of the Association to receive the *Journal of Economic Biology* which had been founded in 1905 by Mr W. E. Collinge.

In 1910 the ninth meeting of the Association was held on 6 and 7 July, in the University of Manchester. Among the papers read to the meeting were "On the place of economic zoology in a modern university", by Prof. S. J. Hickson, and "Wild bird protection", by Mr W. E. Collinge. At this meeting an Associate was elected for the last time. Associates remained on the membership list of the Association until 1914 but, in that year, they seem either to have become ordinary Members or to have discontinued their membership of the Association.

During 1911 meetings were convened at Birmingham University on 6 and 7 April, and in the rooms of the Linnean Society, London, on 7 July. Among the papers at the Birmingham meeting were "The training of economic entomologists", and "The standardization of economic nomenclature", both by Mr H. Maxwell Lefroy; and "The systematic recording of diseases of economic plants", by Dr J. H. Priestley. At the annual meeting in London official business only was transacted.

In 1912 the eleventh general meeting was held in the Royal College of Science, Dublin, on 28 and 29 March. Among the nine papers read were Prof. G. H. Carpenter's Presidential Address on "Biological training for agricultural students", and "Cereal breeding in Ireland", by Mr H. Hunter.

Until about 1912 the general organization and working arrangements of the Association, with secretarial headquarters in Birmingham and meetings held at irregular intervals in various educational centres, had worked fairly smoothly. About that time, however, the feeling became increasingly strong among the Officers and other active Members, that the Association would function more satisfactorily if its headquarters were in London and if its meetings were held at regular and more frequent intervals. Further, owing to difficulties which arose almost inevitably concerning the relationship between the Association and the *Journal of Economic Biology* which was owned privately by Mr Collinge, there was a widespread opinion that the Association should publish a scientific journal of its own.

After considerable discussion, both privately among Members and in Council, a Special Meeting was called in Birmingham on 13 September 1913, and the following resolutions were passed: "The meetings to be quarterly if material allows, and to be ordinarily in London, with one meeting in the Provinces annually. The management of the Association to be centred in London." "This meeting requests the Council to terminate the present arrangement with the Editor and Publisher of the *Journal of Economic Biology* regarding the supply of the *Journal* to Members with a view to the whole question of the relation of the *Journal* and the Association being reconsidered."

The twelfth general meeting was held on 30 December 1913, in the University of Liverpool and, in presenting their seventh Annual Report, the Council "have to report a number of proposed changes which they believe will lead to a renewal of

activity and the continued prosperity of the Association". The changes were in accordance with the resolutions noted above. Some thirty Members and visitors attended this meeting and ten papers were read.

These changes, which amounted to a major revolution in the conduct and policy of the Association, reflected the opinion general among the active workers in the society that the time was ripe for the Association to assume greater responsibility for the development of the science of applied biology and a more independent and prominent place in the organization of science. The Association had become the recognized society for all British workers interested in applied biology, but its provincial organization, and its dependence for the publication of its work on a private journal, were felt to be out of keeping not only with its accepted status but with the general development of the science in the country.

At this time one of the most active Members of the Association was Prof. H. Maxwell Lefroy, a man of unusual energy and vision, and it is largely to his credit that the revolution in the society was carried through successfully.

The transfer of the headquarters of the Association from Birmingham to London made it impossible for Mr W. E. Collinge to continue as Hon. Secretary. To mark their appreciation of his services the Members of the Association presented to Mr Collinge "a handsome piece of plate".

During the autumn and winter of 1913 negotiations were carried on with the Cambridge University Press with a view to the publication of an official journal of the Association. At the thirteenth general meeting of the Association held on 17 and 18 April 1914, in the Zoological Department of the Imperial College of Science, London, the President, Prof. Robert Newstead, announced the success of these negotiations and stated that Members would in future receive the *Annals of Applied Biology*, the "official organ of the Association". About thirty-five Members attended this meeting, forty new Members and two Honorary Members were elected, and eighteen papers were communicated. On the evening of 17 April, an Association Dinner took place in circumstances of considerable anxiety, since a few hours earlier Prof. Lefroy, who had been mainly responsible for its organization, had a serious motoring accident and was in a critical condition.

During the three or four preceding years the Association had made comparatively little progress, but this London meeting, the first to be held in the Imperial College of Science, marked the beginning of a period of renewed activity.

A second meeting was arranged in the Imperial College on 3 July 1914. Sixteen Ordinary Members and one Honorary Member were elected, and nine papers were read to the Association, including "Some aspects of deterioration in plants", by Dr E. J. Butler; "The improvement of Indian sugar canes", by Dr C. A. Barber; and "Insecticides; some considerations from a chemical standpoint", by Mr W. H. Nuthall.

In August 1914 the European war broke out. No further meetings of the Association were held during the year, but a Council meeting took place on Friday, 7 August 1914, and the following extract may be quoted from the Council minutes: "The next meeting was discussed: in view of the exceptional circumstances prevailing, the nation being at war with Germany and Austria as allies of France, Belgium, Russia, and Servia, it was decided to leave to the Secretary to settle, in September, if the proposed meeting be possible or not and for him to act accordingly." Although many Members were absent on active service and others were involved in various war-time activities,

it was decided to keep the Association in being and to maintain the *Annals of Applied Biology*.

A short meeting of the Association was convened on 20 December 1915, in the Imperial College, when eleven Members were present and official business only was transacted.

The next time the Association met was on 21 June 1917. This meeting took place in the Botanical Department of the Imperial College of Science, a Department which, thenceforward, became the unofficial but recognized home of the Association. Thirteen Members and four visitors attended and six communications were made.

In the minutes of a Council meeting held on 21 November 1917, the following appears: "It was proposed, possibly in conjunction with the Imperial Bureau, to make representations to the Minister of Reconstruction pointing out the great importance of Economic Biology and expressing the hope that this will not be lost sight of after the war." A minute of a Council meeting held on 20 March 1918 reads: "A draft of the Memorandum to be sent to the Ministry of Reconstruction was discussed. Dr Marshall said that it had been highly approved of both by Lord Harcourt and Sir Herbert Read."

In 1918 a meeting was called on 21 March; twelve Members and two visitors were present, and three papers were read.

During the war period Members engaged on war service were allowed to become "dormant Members", no subscriptions being required from them. This action and the difficulty of communicating with Members overseas greatly reduced the income of the Association, and the chief endeavour of the Council was directed to keeping the *Annals of Applied Biology* in being. Although the European war terminated in November 1918, conditions were very disturbed and many Members of the Association were not free to participate in its activities. A meeting was, however, held on 11 December 1918, when twenty Members and seven visitors were present, and four papers were communicated.

During the war period the Association had, with considerable difficulty, maintained itself in existence and had continued to publish the *Annals of Applied Biology*. By 1918, however, its activities and vitality had been reduced to a low ebb. The zoological side of the Association had always been the stronger and, in 1918, the botanical Members numbered less than one-third of the total membership. Some of the younger Members, particularly, felt that the Association could be revitalized by changing certain aspects of its conduct and general policy.

In January 1919, Mr W. B. Brierley submitted to the Council a memorandum containing proposals for the reorganization of the Association and the *Annals of Applied Biology*, and for a new orientation of general policy. Certain further proposals made by Prof. Lefroy were of interest in that they involved a change in the status of the Association from that of a scientific society to that of a body empowered by charter to grant some form of Degree or Diploma to those following the profession of applied biologist—on the need for which Prof. Lefroy held very strong views. After considerable discussion Mr Brierley's memorandum and Prof. Lefroy's proposals, together with the views of the Council thereon, were printed and circulated to all Members. The issues were discussed at ordinary meetings of the Association held on 20 March 1919 and 2 and 3 July 1919, and the proposals formulated in the memorandum were adopted.

A first desideratum was to increase the membership, especially on the botanical side, and, at the next meeting of the Association, on 10 and 11 December 1919, the names of sixty-nine botanists were proposed for membership. Five exhibits were shown and three papers were read. Furthermore, previous meetings of the Association had been confined to the reading of individual papers, but at this meeting was initiated the practice of holding symposia. This first symposium was on "The integration of mycological research with practice in agriculture, horticulture, and forestry", Sir Daniel Hall dealing with administration, Prof. V. H. Blackman with teaching, Dr E. J. Russell with agriculture, Mr F. J. Chittenden with horticulture, and Prof. William Somerville with forestry. The symposium was published in full as "Proceedings of the Association" in the *Annals of Applied Biology*.

In the early days of the Association it had been decided to form a library of books and reprints by exchange of the *Annals*, purchase, and donation. As the Association had no official buildings and only limited financial resources, the scheme did not progress. Further, with the founding of the Imperial Bureaux such a library was redundant. In 1920 the scheme lapsed and such literature as had accumulated was deposited with the Imperial Bureau of Entomology, certain duplicates being sent to the Rothamsted Experimental Station.

After 1920 the Association showed slow but steady progress. Six to ten meetings per year were convened during the winter months in London, with an occasional meeting in the Provinces; a field meeting was, with rare omission, held each summer in some research station, botanic garden, or other place of applied biological interest; and frequently, also, a half-day excursion was arranged each autumn to some centre of research in applied biology.

During 1920 three London meetings were held, and visits were paid to the Royal Botanic Gardens, Kew, and to the Rothamsted Experimental Station. Twelve papers were communicated to the Association, and a discussion took place on "The reclamation of waste land". The average attendance at meetings was about seventy-three.

The year 1921 commenced a period of increased activity of the Association. Eight London meetings were arranged, and a day was spent at Reading in the trial grounds of Messrs Sutton and Sons, and in the Agricultural Botanic Garden of Reading University. Ten papers were read before the Association; the retiring President, Sir David Prain, delivered his Presidential Address on "Some relationships of economic biology", and general discussions or symposia were held on the following subjects: "The physiology of the infection process", "Meteorological conditions and disease in plants", "The resistance of the normal and injured plant surface to the entry of pathogenic organisms", and "The importance of scientific research in forestry and its position in the Empire". The average attendance at meetings was about seventy-three.

The period 1919–21 during which the Association was reconstructing itself were years of anxiety and difficulty. Perhaps only the Officers and Council during that period realize fully the debt the Association owes to Sir David Prain, President of the Association from 1919 to 1921. A minute from the Council meeting held on 9 December 1921 may be quoted: "Sir David Prain intimated his desire to surrender the Office of President of the Association. The Council expressed their grateful appreciation of the wise guidance with which the retiring President had conducted the Association through the very difficult years of his term of Office." An equally good friend and wise guide during a further difficult period was Sir E. B. Poulton, President for the years 1922–3.

In 1922 six London meetings were arranged, and a meeting extending over two days was held in Manchester at the Victoria University and the Shirley Cotton Research Institute. Seven papers were read before the Association and general discussions or symposia took place on the following subjects: "Virus diseases in plants and animals", "Sea fisheries research", and "Genetics in relation to applied biology". The average attendance at meetings was about sixty-three.

In 1923, seven London meetings were arranged, and a day was spent at Cambridge visiting the School of Agriculture, Plant Breeding Institute, University Farm, and National Institute of Agricultural Botany. Nine papers were read to the Association and general discussions or symposia were held on the following subjects: "Partial sterilization of soil", "Pathogenic Protozoa in plants and animals", and "The nature of ultramicroscopic viruses". The attendance at meetings averaged about fifty-nine.

Commencing with the year 1924 the Annual Reports of the Council of the Association and the Hon. Treasurer's Statement of Accounts have been published in full in the *Annals of Applied Biology*, part 2 of each volume. The following data have been extracted from these Annual Reports:

(*a*) *Presidential Addresses*

1924—Prof. E. B. Poulton, "The relation of pure and applied biology"; 1926—Prof. V. H. Blackman, "Recent work in plant physiology and its relation to applied biology"; 1928—Mr J. C. F. Fryer, "Legislation in England against diseases and pests of plants"; 1930—Dr E. J. Butler, "Some aspects of the morbid anatomy of plants"; 1932—Dr A. D. Imms, "Temperature and humidity in relation to insect control"; 1934—Prof. W. B. Brierley, "Some viewpoints of an applied biologist"; 1936—Dr T. Goodey, "Some applied biological aspects relating to plant parasitic nematodes"; 1938—Dr J. Henderson Smith, "Some recent development in virus research".

(*b*) *General discussions and symposia*

1924—"Vegetative propagation"; "Cold storage problems"; "Genetics and the stock-breeder"; General principles that should underlie government action regarding fungicides and insecticides: 1925—"Research on wart disease of potatoes"; "The use of sulphur as a fungicide"; "The place of the systematist in applied biological work": 1926—"Forestry in relation to science"; "Crown-gall of plants": 1927—"Plant alkaloids"; "Research in applied biology at the South Eastern Agricultural College, Wye"; "Agricultural problems in tropical Africa"; "Foot and mouth disease of cattle": 1928—"Work of the Plymouth Marine Laboratory"; "Work of the Forest Products Research Laboratory, Princes Risborough"; "Relation of environmental conditions to disease in plants": 1929—"Work of the Empire Marketing Board"; "Agricultural science in Australia"; "Work of the National Institute for Research in Dairying, Reading"; "Research on infestation of stored products"; "The incidence and control of apple scab": 1930—"Biological control of injurious insects and weeds"; "Nutrition of fruit trees"; "Factors influencing yield of cereal crops"; "Purification of waste waters from sugar beet factories": 1931—"Biological races and their significance in evolution"; "Economic applications of microbiology"; "Training of biologists for economic posts"; "Laboratory tests of fungicides": 1932—"Decay of stonework"; "Fungal deterioration of stored products and dairy produce"; "Decomposition of plant materials": 1933—"Pests of mushrooms"; "Physiological

disorders of glasshouse crops and fruit trees": 1934—"Plant pathological problems in the tropics": 1935—"Nitrification processes in the soil"; "Sawfly problems"; "Glasshouse problems at the Experimental and Research Station, Cheshunt"; "Insect population studies": 1936—"Problems raised by the woolly aphis of the apple"; "Effect of various conditions on the resistance of apples to fungal attack": 1937—"Recent work on the death-watch beetle, *Xestobium rufovillosum*"; "Recent developments in fumigation"; "The wireworm problem": 1938—"Chemical weedkillers"; "Fresh-water biology and its applications"; "Apple canker".

(*c*) *Provincial and field meetings and visits*

1924—Leeds University; Wembley Exhibition, London: 1925—Edinburgh University; nurseries of Messrs Bunyard, Maidstone: 1926—Experimental and Research Station, Cheshunt: 1927—South-Eastern Agricultural College, Wye; Imperial Institute, London: 1928—Long Ashton Research Station, Bristol; Royal Botanic Gardens, Kew: 1929—London Docks and Warehouses: 1931—Parasite Station of the Imperial Institute of Entomology, Farnham Royal: 1932—Biological Field Station of the Imperial College of Science, Slough: 1933—Reading University: 1934—Wellcome Physiological Research Laboratories, Beckenham; London School of Hygiene and Tropical Medicine: 1935—East Malling Research Station; Lister Institute of Preventive Medicine, London: 1936—Royal Horticultural Society's Gardens, Wisley; Wellcome Museum of Medical Science, London: 1937—Experimental and Research Station, Cheshunt; Gardens of the Zoological Society of London: 1938—Forest Products Research Laboratory, Princes Risborough; Gaumont British Film Studios, London.

From 1917 until 1935 all ordinary meetings of the Association in London were convened in the botanical lecture theatre of the Imperial College of Science. Council meetings are still held in the Botany Department but, since 15 November 1935, nearly all ordinary meetings of the Association have taken place in the metallurgy lecture theatre of the Imperial College. Until 1931 the London meetings were held on Friday afternoons but, in that year, the present practice was adopted of holding two full-day meetings and two half-day meetings per year. During 1924 Members first met for tea and social intercourse after the meetings.

From its inauguration in 1904 the Society had been known as the *Association of Economic Biologists*. After considerable discussion and the taking of a referendum of all Members, it was decided at an extraordinary general meeting held on 24 February 1934, to change the original title to the *Association of Applied Biologists*.

II. The *Annals of Applied Biology*

When the Association of Economic Biologists was inaugurated in November 1904 the publication of an official journal was not envisaged. It was decided to issue annual *Proceedings of the Association* containing the Annual Reports of the Council and the Hon. Treasurer, notices of the meetings, and abstracts of the papers communicated to the society. Four such *Proceedings* were published for the years 1905–8.

In 1905 Mr W. E. Collinge, Hon. Secretary of the Association, founded the *Journal of Economic Biology*, part 1 of vol. 1 appearing under date of 15 November 1905. The following extracts may be quoted from an Editorial: "It has for some time been apparent that workers in Economic Biology have found difficulty in obtaining

publication of their papers, and particularly so if good illustrations were required. With the foundation of an Association of Economic Biologists in the United Kingdom, such papers will naturally increase in number, and whilst this body is able to deal with the publication of the smaller papers larger ones requiring carefully executed plain or coloured lithographic plates are still unprovided for. It is intended in this journal to offer a medium for such work."

The *Journal of Economic Biology* belonged to Mr W. E. Collinge and not to the Association, and the Association, as such, had no financial or editorial interest in the publication. The *Journal* was, however, quite generally regarded as the unofficial organ of the Association. It was edited by Mr W. E. Collinge, Hon. Secretary of the Association, with the co-operation of Prof. A. H. R. Buller, Prof. G. H. Carpenter, Mr Robert Newstead, and Mr A. E. Shipley, one a Vice-President of the Association, and all active Council Members of the Association. Furthermore, many of the papers read to the Association, including some of the Presidential Addresses, were published in the *Journal of Economic Biology*.

In 1908 the Council of the Association entered into an agreement with Mr Collinge whereby he agreed to supply the *Journal* to Members of the Association and "to publish the various papers read before the Association, if the same should be deemed worthy of publication". The financial and general control of the *Journal* remained, as hitherto, in the hands of its proprietor, Mr W. E. Collinge. The only changes in the *Journal* were that Prof. Percy Groom, newly elected to the Council of the Association, was added to the Editorial Board and that the *Proceedings of the Association*, hitherto published annually as separate pamphlets were, from 1909 to 1912, published in the *Journal*.

The annual subscription to the Association was 10*s*. 6*d*. but, in accordance with the new arrangement whereby Members received the *Journal of Economic Biology* free, the subscription was raised to 21*s*. The arrangement came into force as from January 1910, with vol. v of the *Journal*, and continued until 1913.

This arrangement, although convenient at the time, did not prove satisfactory. It became obvious that it was not to the best interests of the Association that its *Proceedings* and scientific papers should be published in a journal unofficially sponsored by the Association but over which the Association possessed no editorial or financial control. At a special general meeting of the Association held on 12 September 1913, it was decided that the arrangement with Mr Collinge should lapse with part 4, vol. VIII, December 1913, of the *Journal*, and that the Association should manage its own official publication.

The *Journal of Economic Biology* appeared during 1914 under the Editorship solely of Mr W. E. Collinge, and, at the end of 1915, was discontinued. In 1916 it appeared as the *Journal of Zoological Research*, edited by Mr W. E. Collinge, but finally disappeared with no. 4, vol. III, December 1918.

The last *Proceedings of the Association* to be published in the *Journal of Economic Biology* appeared in part 4, vol. VII, December 1912.

The decision of the Association to publish a scientific journal of its own required faith and courage, since the financial position of the Association was far from strong, and the membership had actually dwindled from 132 in 1909 to 105 in 1913. The Council's Annual Report presented to the twelfth general meeting on 30 December 1913, records that "The total receipts up to December 1913 amounted to £66. 12*s*. 0*d*.

whilst the total expenditure for the same period amounted to £88. 13*s*. 3*d*., leaving a balance in the hands of the Hon. Treasurer of £100. 7*s*. 1*d*. There is also an outstanding balance of £43. 13*s*. 0*d*. for subscriptions owing". Nevertheless, during 1913, negotiations were entered into with the Cambridge University Press and, in May 1914, part 1, vol. I of the *Annals of Applied Biology* appeared.

Not only the Association but the science of applied biology are deeply in debt to Prof. Maxwell Lefroy whose energy and vision were largely responsible for the successful inauguration of the *Annals*.

The *Annals of Applied Biology* was the property of the Association, a scientific journal over which the Association possessed full editorial and financial control. The Hon. Secretary of the Association, Prof. H. Maxwell Lefroy, was appointed Hon. Editor, and in conducting the new *Annals* he was assisted by an Editorial Committee consisting of Prof. B. T. P. Barker, Dr S. E. Chandler, Mr F. J. Chittenden, Prof. F. W. Gamble, Prof. Percy Groom, Dr A. D. Imms, Prof. Robert Newstead, and Prof. J. H. Priestley.

In an Editorial to the first number of the *Annals* Prof. Maxwell Lefroy indicated the general policy to be pursued, and extracts from this may be quoted: "The Association of Economic Biologists was founded ten years ago and commences herewith the publication of a journal devoted to the special interests of its Members. During this period its scope has broadened and the *Annals of Applied Biology* is intended to cover the ground in Applied Biology which is not now covered by special journals such as those dealing with agricultural science, parasitology, genetics, and medical science." "All papers which bear on the scientific problems of applied biology will be welcome; we have no place for purely systematic work which is amply provided for elsewhere, nor for faunistic work as such." "We hope to attract not only the more solid scientific contributions but also notes of progress, of interesting achievements, of practical problems, as they present themselves to Members in the various parts of the Empire."

From 1914 to 1924 (vols. I–XI) the *Annals* was published somewhat irregularly as material became available. Two parts of each volume appeared as separate issues, and the two remaining parts as a double number.

With the completion of vol. II of the *Annals* issued in April 1916, Prof. H. Maxwell Lefroy relinquished the Hon. Editorship and Mr E. E. Green, lately Government Entomologist in Ceylon, was invited by the Council to undertake the work. There was no change in editorial policy or in the Editorial Board but, in December 1919, the double number containing parts 2 and 3 of vol. VI appeared under the Hon. Editorship of Mr E. E. Green "with the assistance of the Council".

With the completion of part 4, vol. VIII, February 1921, Mr E. E. Green relinquished the Hon. Editorship and the Council of the Association decided to effect certain rearrangements in the conduct and policy of the *Annals*. Mr W. B. Brierley was invited to become Hon. General and Botanical Editor, and Mr D. Ward Cutler Hon. Zoological Editor. A "Publications Committee" was appointed consisting of the Hon. Treasurer, two Council Members, and two Ordinary Members (A. D. Imms, V. H. Blackman, E. E. Green, A. W. Hill, R. T. Leiper).

Since 1921 there has been no change in the Hon. General and Botanical Editorship of the *Annals*. Mr D. Ward Cutler remained Hon. Zoological Editor until 1932, when he was succeeded by Prof. J. W. Munro in 1933, and by Mr C. T. Gimingham in 1934.

Vol. I of the *Annals* was published in May and July 1914, and January 1915, i.e. it contained investigations completed prior to the outbreak of the war in August 1914. The volume comprised 406 pages and 27 plates. The early months of the war left a sufficient number of workers to carry on, and in the minutes of a Council meeting held on 27 January 1915, the following appears: "The question of the future of the *Annals of Applied Biology* was discussed. It was decided to issue four numbers of reduced size." Parts 1, 2 and 3 of vol. II were issued in May and July 1915, but in the minutes of a Council meeting held on 29 October 1915, the following appears: "It was decided to suspend publication of *Annals*, II, 4, till the General Meeting had expressed an opinion, unless it was found that there were funds sufficient on this year's (1915) subscriptions and sales to admit of its immediate issue." Fortunately, the Royal Society of London generously made a grant of £50 towards the publication of the *Annals*. Part 4 was, therefore, issued in April 1916, the total volume containing 292 pages and 39 plates.

Difficulties, however, continued to increase. Vol. III of the *Annals*, published June 1916, and January and April 1917, was reduced to 204 pages and 24 plates but, even so, it cost more than the depleted funds of the Association could stand. Again, the Royal Society of London generously granted a sum of £50 towards publication expenses, and vol. IV, comprising 239 pages and 12 plates, was issued September and December 1917, and March 1918. The Association is greatly indebted to Prof. Newstead, then President, since it was in no small measure due to his exertions that the claims of the Association were brought successfully to the notice of the Royal Society.

With the *Annals* to carry on, finance was the chief preoccupation of the Council during these years, but in the minutes of a Council meeting held on 21 November 1917, the following appears: "It was further proposed by Dr Marshall and seconded by Dr MacDougall that in view of the anticipated balance at the end of the year, no application for a further grant to the Royal Society should be made. This was also approved." A minute of the Council meeting held on 16 October 1918, reads: "The Treasurer made a statement as to the satisfactory financial position of the Association. ...After some discussion it was not considered advisable to invest the £100 now held in reserve or to raise the price of the *Annals*." Vol. V of the *Annals*, comprising 281 pages and 11 plates, was issued July and October 1918, and April 1919.

With the release of men from the army or war services after November 1918, the demand upon the *Annals*' space began to increase. Further, the Association for the first time in many years possessed a little money in reserve. Accordingly, vol. VI of the *Annals*, published September and December 1919, and April 1920, was enlarged to 356 pages and 10 plates. The increased costs of production of this volume caused disquiet in the mind of the Hon. Treasurer. At a Council meeting held on 5 November 1919, he presented a statement "respecting the financial position of the *Annals*", suggested certain economies, and "Recommended that an attempt should be made to obtain a grant in aid from the Royal Society or from the Development Commission of the Board of Agriculture". It was decided to apply for a sum of £200. At a general meeting of the Association held on 4 June 1920, the "Chairman announced that the Development Commissioners had recommended a grant to the Association up to £200 and that this recommendation had been sent to the Treasury".

This generous grant practically saved the *Annals* and the Association is all the more grateful for its receipt since the Development Commissioner's policy at that time

was contrary to making grants for publication. The Association is deeply indebted to Sir David Prain, then President, and Mr J. C. F. Fryer, then Hon. Treasurer, for their successful efforts in obtaining this grant.

The money was received, and vol. VII of the *Annals* issued September and December 1920, and February 1921, was enlarged to 431 pages and 26 plates, three of the latter being in lithograph and one in colour. This was by far the largest and most opulent volume of the *Annals* the Association had yet produced and the expenses of publication were correspondingly large, especially as general costs of production were rising steeply during this period.

Parts 2 and 3, the double number of vol. VII, which contained 204 pages and the four special plates, alarmed the Hon. Treasurer and, at a Council meeting on 10 December 1920, "The question of the publication of the *Annals of Applied Biology* was discussed at some length and a sub-committee was appointed to consider the matter and report to the next Council meeting". Their report was presented at a Council meeting on 28 January 1921. Among their recommendations were the raising of the annual subscription to the Association from 21*s*. to 25*s*. and of the subscription price of the *Annals* from 33*s*. 6*d*. to £2, a reduction in author's free reprints from 50 to 25, and "That for the present no illustrations in the *Annals of Applied Biology* other than text-figures be paid for by the Association save under exceptional circumstances". "Material financial retrenchment" was imperative if the Association were to remain solvent, and it was decided to raise a "Publications Fund for the purpose of bridging over the difficulty of carrying on the *Annals of Applied Biology*".

Vol. VII was the last produced under the Hon. Editorship of Mr E. E. Green and the new Hon. Editors, Mr W. B. Brierley and Mr D. Ward Cutler, elected in December 1920, were faced with the task of producing the *Annals* in the Association's greatly reduced circumstances. By dint of stringent economy and drastic cutting, and the receipt of "Grants in aid of publication" from eight of the seventeen authors contributing manuscripts, vol. VIII was produced and was issued June, August, and November 1921. This volume, which contained only 219 pages and 4 plates, was the nadir of the *Annals of Applied Biology*. In an "Editorial Note" to part 1 of vol. VIII of the *Annals* the new Hon. Editors outlined certain of the economies considered necessary but stated, "It is hoped that the reductions outlined above will only be operative for the current year since it is anticipated that in 1922 it will be possible to revert to improved conditions".

During 1922 financial conditions, although still very difficult, were slightly better than in the previous period, but this improvement was offset by the fact that, with the return of the country to normal, more scientific work was carried out and more manuscripts were submitted for publication in the *Annals*. The "Publications Fund" had reached some £70 and, by the exercise of strict economy, and ruthless editorial demands for "Grants in aid of publication", vol. IX of the *Annals* was produced. Twenty-four papers were published and the *Annals*, issued April, June, and November 1922, contained 359 pages and 14 plates.

The Association had survived the crisis and maintained its *Annals*, and, although passing years saw slight changes of fortune, the progress of the Association and its *Annals* has been well maintained. Vol. X, published February, July, and December 1923, was increased to 454 pages and 22 plates, and vol. XI, issued April, July, and October 1924, still further enlarged to 519 pages and 16 plates. In this volume was

initiated the practice of publishing the Hon. Treasurer's "Statement of Accounts" for the preceding year. The Statement for the year ending 31 December 1923 showed a cash balance of £101. 18*s*. 5*d*. and the sum of £400 invested in Savings Certificates, which has remained as the nucleus of a reserve fund.

So far two separate numbers and one double number of the *Annals* had been published somewhat irregularly each year. During 1924 the Hon. Editors made arrangements with the Cambridge University Press for the issue of four separate parts per year and, in 1925, the parts of vol. XII appeared in February, May, July, and November. The volume, the largest yet published by the Association, contained 549 pages and 18 plates.

Commencing with 1926 the four parts of the *Annals* have, with one or two exceptions only, and then merely by a matter of a few days, appeared regularly in the months of February, May, August, and November. Furthermore, unless papers were unusually long or required an unusual number of plates, no request has been made for "Grants in aid of publication", and no subsidy has been necessary from outside sources. Vol. XIII (1926) comprised 645 pages and 20 plates. Vols. XIV–XXI (1927–34) had an average size of 690 pages and 36 plates. Vol. XVII, containing 810 pages and 50 plates, was rather a special effort in view of the holding of the Fifth International Conference of Botany at Cambridge in 1930.

Commencing about the year 1933 the number of manuscripts submitted to the Hon. Editors began to increase and this resulted in delay in the publication of research. Vol. XXII (1935) was, therefore, increased to 820 pages and 35 plates. Part 2 of this volume contained a paper of 82 pages by H. Martin on "The standardization of petroleum and tar oils and preparations as insecticides" which the Council reprinted for sale as a separate issue. Vol. XXIII (1936) was increased to 921 pages and 39 plates; and Vol. XXIV (1937) to 940 pages and 51 plates. Vol. XXIV of the *Annals* was the largest ever published by the Association and contained 59 scientific papers, a "Proceedings of the Association" of 15 pages, reviews of 44 books, and the Reports of the Council and Hon. Treasurer.

As the official scientific journal of the Association of Applied Biologists the *Annals of Applied Biology* is devoted to the special interests of Members of the Association. The great majority of the papers published in the *Annals* since its commencement in 1914 have been contributed by Members or by scientific workers who later became Members. Not infrequently, however, papers by non-members and by workers in various countries outside the British Empire have been accepted.

In the first 25 volumes of the *Annals of Applied Biology* (1914–38) the total number of papers, including "Proceedings", notes, and longer obituary notices, is 960. These may roughly be classified as follows:

General applied botany (including non-parasitic diseases)	131
Mycology and fungal diseases	160
Bacterial diseases	50
Viruses and virus diseases	108
General applied zoology	26
Entomology and insect pests	250
Plant protection (fungicides, insecticides, etc.)	99
Microbiology (of food, soil, etc.)	62
Helminthology and nematode diseases	20
Apparatus (botanical and zoological)	10
General (botany, zoology, obituary notices, etc.)	44

Earlier volumes of the *Annals* contained occasional book reviews, but in 1924 the Hon. Editors, in response to suggestions from workers in overseas parts of the Empire, enlarged this service of the *Annals*. Commencing with Vol. XI the average number of book reviews per volume has been 18.

Free copies of the *Annals of Applied Biology* are supplied as follows:

Copyright Libraries. 6 copies.

Syndics, Cambridge University Press. 1 copy.

Botany Department, Imperial College of Science and Technology, London; in grateful recognition of its hospitality. 1 copy.

During the whole period of its existence the *Annals of Applied Biology* has been produced and published for the Association by the Cambridge University Press. No praise can be too high for the technical efficiency or artistry of its production, or for the consummate skill shown by the Press readers.

III. Conclusion

The development of the Association shows certain stages which may be related to more general developments in the country.

Prior to 1904 a mere handful of workers in Applied Biology was scattered in various appointments throughout the United Kingdom. There was little opportunity or encouragement for research in this field, since few facilities existed, and the emphasis in Universities and other educational institutions was laid primarily on pure science. The only agricultural research institution in the country was the Rothamsted Experimental Station where biology had not gained a foothold.

The Association was founded in November 1904. The period 1905 to about 1909 was one of rapid growth of the Association, and its practice of holding meetings in various educational centres throughout the United Kingdom—Birmingham, Liverpool, Cambridge, London, Edinburgh, Oxford, Manchester, Dublin—must have had great propaganda value for the subject of applied biology, and, in large part, may be held responsible for the steadily increasing recognition of the importance of the subject.

This recognition received practical outcome with the passing of the Development Fund Act in 1909, whereby a sum of two million pounds was set aside for the development of agricultural education, and particularly of agricultural research. During the next five years the scheme of agricultural research institutes, in principle almost as it exists to-day, came into being. The scheme had, however, barely come into active operation when the war broke out in August 1914.

Until about 1909 the Association had been finding itself, energizing and proselytizing in all directions. The period 1909 to about 1913 was also one of considerable activity in the Association, but this activity was directed inwardly rather than to any specific external new developments. The Association reorganized itself, settled down in London, and decided to produce the *Annals of Applied Biology*.

With 1914 the schemes of agricultural and horticultural research in the country began to function adequately and there was a great spirit of optimism throughout the whole field of applied biology. This was reflected in the increased membership and activity of the Association, which seemed about that time to assume a new lease of life, and in the founding of the *Annals of Applied Biology*. In August, came the European war and this promise came to an abrupt end.

During the war years the Association maintained itself in existence but it became a society with one preoccupation—the production of the *Annals*. It was not until after 1919 that conditions had returned sufficiently to normal to enable the Members to come together and, in the meantime, their sole link was the *Annals*.

The period 1919 to 1922–3 was one of slow recovery and general reorganization in the agricultural research schemes of the country. With the repeal of the Corn Production Acts in 1921 a sum of £850,000, to be spread over five years, was appropriated for agricultural education and research. This permitted the adequate development of schemes which had fallen into abeyance and the inauguration of new schemes and, about 1922–3, the effects began to be seen in an increased number and activity of research workers and an expanding volume of research. During this period the Imperial Bureau of Entomology, founded some years earlier, resumed and increased its activity, the Imperial Bureau of Mycology came into being, an Institute of Plant Pathology was founded at the Rothamsted Experimental Station, the Ministry of Agriculture's Plant Pathological Laboratory was established at Harpenden, and other important developments occurred.

In the Association the period 1919 to 1922–3 was one of great difficulty. In the winter of 1918–19 the Association had reached its lowest ebb and the *Annals* was almost defunct, but, during 1919–21, the Association was reorganized. It survived the crisis and by 1922–3 was in a condition to meet the accelerating demands for regular scientific meetings and for increased facilities for publication.

In the country the next six years (1923–8) was a period of expansion. The agricultural and horticultural research institutes developed rapidly, and great encouragement was received from the Imperial Agricultural Conference of 1926, and the establishment of that fairy Godmother, the Empire Marketing Board, which initiated and developed numerous research schemes, including that of virus diseases of plants. Additional Imperial Bureaux were established and began to make their influence felt. The Department of Scientific and Industrial Research expanded rapidly.

In the Association the period 1923–8 was one of great activity; numerous and successful meetings were held, and the *Annals of Applied Biology* from a volume of 359 pages and 14 plates in 1922 expanded at almost a steady rate per year to a volume of 707 pages and 39 plates in 1928. To take virus research as one example. In the fourteen volumes of the *Annals* published from 1914 to 1927 there were only nine papers on viruses and virus diseases. About 1927 the virus research schemes came into full working order, and, in the eleven volumes of the *Annals* published from 1928 to 1938, the papers on this subject number about one hundred.

Since 1928 the Association and its *Annals* have experienced fluctuations of fortune but the general trend of membership, activity at meetings, and publication has been in an upward direction. There have been no marked changes in the constitution of the Association or, save in the steady widening of its interests, new and noteworthy developments in its general activities. Perhaps the most striking development has been the increase in size and scientific value of the *Annals of Applied Biology*.

Appendix

The following Members have served as Officers of the Association of Applied Biologists in the capacities and for the periods noted:

I. *Presidents*

Mr Fred V. Theobald	1904–1906	Mr J. C. F. Fryer	1926–1927
Sir A. E. Shipley	1907–1909	Dr E. J. Butler	1928–1929
Prof. G. H. Carpenter	1910–1913	Dr A. D. Imms	1930–1931
Prof. Robert Newstead	1914–1917	Prof. W. B. Brierley	1932–1933
Sir J. B. Farmer	1918	Dr T. Goodey	1934–1935
Sir D. Prain	1919–1921	Dr J. Hendersom Smith	1936–1937
Sir E. B. Poulton	1922–1923	Mr C. T. Gimingham	1938–
Prof. V. H. Blackman	1924–1925		

II. *Vice-Presidents*

Sir P. Manson	1905–1915	Dr E. J. Butler	1924–1925
Sir A. E. Shipley	1905–1907, 1914–1919	Dr A. D. Imms	1924–1927
Prof. W. Somerville	1905–1909, 1920	Dr G. H. Pethybridge	1926–1927
Mr Fred V. Theobald	1907–1910	Dr J. Waterston	1928–1930
Sir E. B. Poulton	1909–1913	Prof. F. T. Brooks	1928–1929
Sir J. B. Farmer	1910–1913	Prof. W. B. Brierley	1930–1931
Prof. G. H. F. Nuttall	1910–1913	Dr W. R. Thompson	1930–1932
Sir Ronald Ross	1910–1911	Mr A. D. Cotton	1932–1934
Prof. S. J. Hickson	1911–1920	Prof. W. Brown	1933, 1935
Prof. G. H. Carpenter	1914–1920	Prof. J. W. Munro	1933
Prof. R. Stuart MacDougall	1914–1919	Dr C. B. Williams	1934–1935
Prof. G. Stanley Gardiner	1917–1920	Dr R. C. Fisher	1936
Mr A. G. L. Rogers	1920–1921	Dr S. P. Wiltshire	1936
Sir G. A. K. Marshall	1920–1922	Mr C. T. Gimingham	1937
Prof. V. H. Blackman	1922–1923	Dr H. Martin	1938
Sir E. J. Russell	1923	Dr H. Wormald	1938

III. *Hon. Treasurers*

Mr Herbert Stone	1904–1909	Dr A. D. Imms	1920–1930
Prof. R. T. Leiper	1910–1913	Dr J. Henderson Smith	1931–
Mr J. C. F. Fryer	1914–1919		

IV. *Hon. Editors*

Prof. H. Maxwell Lefroy	1914–1915	Prof. J. W. Munro (Zoological)	1933
Mr E. E. Green	1916–1920		
Prof. W. B. Brierley (General and Botanical)	1921–	Mr C. T. Gimingham (Zoological)	1934–
Mr D. Ward Cutler (Zoological)	1921–1932		

V. *Hon. Secretaries*

Mr W. E. Collinge	1904–1913	Prof. S. G. Paine	1923–1926
Mr W. G. Freeman	1910	Dr T. F. Chipp	1927
Dr S. E. Chandler	1911–1913	Prof. J. W. Munro	1928–1932
Prof. H. Maxwell Lefroy	1914	Prof. W. Brown	1928–1932
Prof. P. Groom	1915–1916	Prof. R. H. Stoughton	1933–1935
Dr S. A. Neave	1917–1921	Mr G. Fox Wilson	1933–
Prof. W. B. Brierley	1919–1922	Mr W. P. K. Findlay	1936–
Dr J. Waterston	1922		

Prof. F. V. Theobald. President 1904–1906

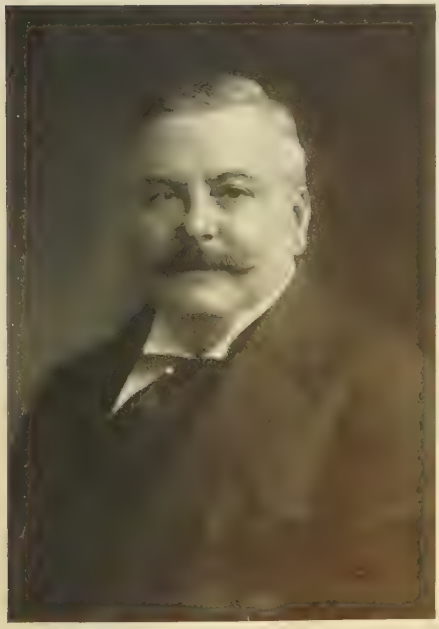

Photo. Elliott & Fry

Sir Arthur E. Shipley. President 1907–1909

Photo. Gardiner

Prof. G. H. Carpenter. President 1910–1913

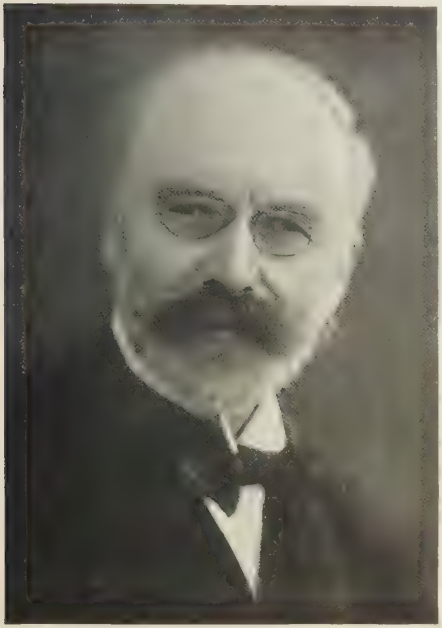

Photo. Elliott & Fry

Prof. Robert Newstead. President 1914–1917

Photo. Lafayette

Sir John B. Farmer. President 1918

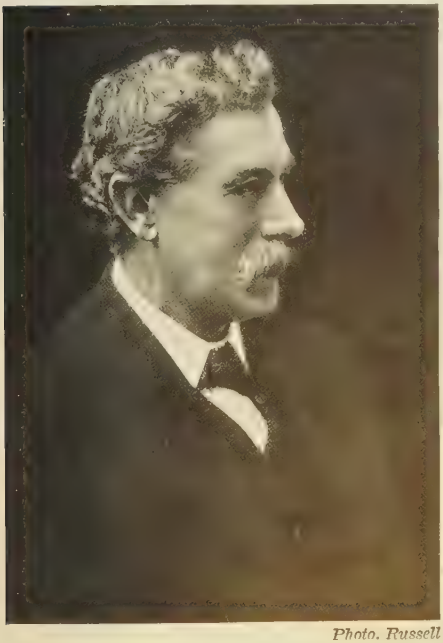

Photo. Russell

Sir David Prain. President 1919–1921

Photo. Lafayette

Sir Edward B. Poulton. President 1922–1923

Photo. Elliott & Fry

Prof. V. H. Blackman. President 1924–1925

Mr J. C. F. Fryer. President 1926–1927

Photo. Elliott & Fry

Dr E. J. Butler. President 1928–1929

Photo. Lafayette

Dr A. D. Imms. President 1930–1931

Photo. Corbett

Prof. W. B. Brierley. President 1932–1933

Dr T. Goodey. President 1934–1935

Photo. Elliott & Fry

Dr J. Henderson Smith. President 1936–1937

Mr C. T. Gimingham. President 1938–

Honorary Members of the Association

A. *Past Honorary Members*:

	Date of election		Date of election
Prof. R. Blanchard	1905	Prof. A. Berlese	1905
Dr A. Looss	1905	Prof. G. Cuboni	1914
Prof. G. L. Neumann	1905	Prof. A. Railliet	1914
Prof. J. Ritzema-Bos	1905	Prof. W. Johannsen	1921
Prof. N. A. Cholodkovsky	1905	Prof. M. W. Beijerinck	1925

B. *Honorary Members*, 1938:

Dr L. O. Howard	1905	Prof. Chevalier	1928
Dr A. D. Hopkins	1905	Prof. P. Silvestri	1928
Prof. P. Marchal	1914	Prof. N. I. Vavilov	1929
Prof. L. R. Jones	1923	Prof. E. Gäumann	1934
Prof. N. H. Nilsson-Ehle	1923	Dr B. P. Uvarov	1934
Prof. O. Appel	1924	Prof. K. Escherich	1934

Membership of the Association

Year	Number	Year	Number
1905	68	1923	255
1906	80	1924	257
1907	97	1925	243
1908	129	1926	241
1909	132	1927	255
1910	118	1928	283
1911	104	1929	289
1912	104	1930	301
1913	105	1931	303
1914	?	(Members in arrears removed)	
1915	?	1932	252
1916	?	1933	285
1917	?	1934	291
1918	?	1935	308
1919	130	1936	310
1920	208	1937	314
1921	247	1938	326
1922	246		

NOTES ON THE PLATES

With the exception of Prof. G. H. Carpenter the portraits are approximately contemporaneous with the Presidential terms of Office.

No early photograph of Prof. Carpenter was available and his portrait is reproduced from a photograph taken in 1938.

I am greatly indebted to Elliott & Fry, Ltd., Lafayette, Ltd., and J. Russell & Sons, for permission to reproduce portraits taken by them.

I also wish to express my gratitude to those who have helped me to complete the Presidential portrait gallery.

REVIEWS

Handbuch der Pflanzenkrankheiten. Herausgegeben von Prof. Dr O. Appel. Bd. VI. *Verhütung und Bekämpfung der Pflanzenkrankheiten.* 2 Lief. Pp. 289–576. Berlin: Paul Parey. 1938. 16.60 Rm.

Dr Appel has found it necessary to issue the comprehensive textbook on Plant Protection which he has added, as vol. VI, to the original Sorauer, in parts, of which this is the second. The first instalment was reviewed in the *Annals*, 1937, **24**, 675. The volume will be completed in four to five such parts which cannot be purchased singly. Consequently the second part opens in the middle of a discussion by Dr W. Trappmann on the use of mechanical barriers for crop protection. He continues to describe, in 44 pages, other physical methods whereby damage by pest or weather may be prevented or by which pests may be trapped. The subject is treated so completely and thoroughly that it may be deduced that this chapter was written towards the close of 1935 but it is a pity that the opportunity of including some references up to 1937 was not used to insert post-1935 work upon other sections, e.g. the use, in eelworm control, of chemicals to stimulate emergence from the encysted stage.

Chemical methods of plant protection come next under review, insecticides and fungicides occupying the greater part (210 pages) of the book. The chemistry of these materials is dealt with by Drs G. Hilgendorff and W. Fischer and the biological application by Drs W. Trappmann, W. Tomaszewski and A. Winkelmann. Each group is described systematically and all information of possible value to the applied biologist is assembled with full references. Upon controversial subjects such as the chemistry of Bordeaux mixture or the action of the copper fungicides, each succeeding hypothesis is mentioned but mature criticism in the text indicates those of practical significance. The many misprints, especially in the titles of non-German articles, are irritating rather than misleading. As the citations appear to have been checked, it is difficult to understand how the spelling errors survived.

The comprehensiveness and richness of this section contrasts with the paucity of sign-posts to the information available. No index is provided, nor is the table of contents appearing in a separate advertisement leaflet helpful since it does not always coincide with the author's arrangement. Thus, derris and other pest control materials of vegetable origin are listed as subsection 7 of nicotine (p. 495) whereas the authors treat this group in section 6 (p. 504). Nor is good use made of the arrangement of the subject matter to facilitate reference. Insecticides and fungicides are classed as inorganic or organic, the inorganic materials being arranged upon the metal-metalloid —non-metal basis of the old-fashioned class books. A biologist trying to find the thallium rat poisons would never think of looking between the sections headed "Bleiverbindung" and "K-, Na-...Ce-Verbindung". Even the chemist would be lost among the organic compounds, arranged on no apparent plan. The most helpful approach is probably that based on function, a scheme which has been partly adopted in discussing supplementary materials. Finally, the device of systematic and distinctive headings has been poorly used. Thus, the reader might well think that the chapter headed "(*b*) Biologische Prüfung von Pflanzen- und Vorratschutzmitteln" was but a subsection of the previous subject "3. Farb-, Riech-,...Warnstoffe".

The criticism that it is difficult to use the book, in its present form, for purposes of reference has been stressed purposely since it is perhaps not too late to provide an index in which the chemicals are grouped according to use. The authors must be permitted full scope in the indexing of their sections for the preparation of the final part, with which a subject index is promised.

The biological methods of testing insecticides and fungicides are next discussed, in 20 pages, by Drs A. Winkelmann and H. Klinger. It is apparent that the subject matter employed by these authors does not go far beyond their laboratory at Dahlem.

Much of it has already appeared in the "Mitteilungen aus der Biologischen Reichsanstalt" and the present section is a useful supplement to Heft 55 of that publication. The opportunity for reviewing other methods and of displaying the value of biological assay in the analysis of the mode of action of insecticides and fungicides or in the development of new materials has not been taken. Surely the authors have heard of the Peet-Grady method, of Campbell's sandwich method, of the work of Tattersfield and his colleagues, to mention but a few of the many omissions concerning insecticides only. Even reference to their compatriot Gerhard Peters is not made although the authors must often have consulted his monograph *Chemie und Toxikologie der Schädlingsbekämpfung*. Nor is the interpretation of the results of biological tests referred to beyond the tabulation of empirical toxicity values of this type: 0 = no eating, I = traces of eating, etc.; space which could well be replaced by mention of the statistical methods due to Henderson Smith, Bliss, O'Kane and others.

The book closes with 15 pages of the section upon physical and chemical methods of testing pest control materials by Drs G. Hilgendorff and W. Fischer who adopt a welcome return to the completeness and comprehensiveness of earlier chapters. It is however disturbing to note that, whereas four closely printed pages are insufficient to describe the quantitative estimation of copper, but one short paragraph is devoted to surface tension, a property employed to evaluate the wetting and spreading powers of spray fluids. But without knowledge of the full contents of Part 3 of the volume, it would be dangerous to suggest that a lack of sense of proportion is apparent in this chapter.

H. MARTIN.

Third International Congress of Comparative Pathology. Athens, 1936. *Reports*, vol. I, part 2. Section of Plant Pathology. Pp. 1–274. Athens: Éditions "Flamma". 1936 (received July 1938).

The present part is devoted to the subject of "Immunity in Plants", and contains papers read to the Congress by Butler, Dufrenoy, Gäumann, Carbone, Magrou, Politis, Reed, Riker, Brenchley, Brooks, Fahmy, Rischkow, Salaman, K. M. Smith & Doncaster, Savulescu, Stakman & Hart, and Humphrey. In an *Extrait du volume des Comptes Rendus* (pp. 35), there are further papers on the subject by Boivin *et al.*, Cavadas, Pinoy, Saregamni & Cortzas, and Apostolides. The papers are in English, French, Italian, and German, conclude with a French résumé, and many possess bibliographies.

The papers cover a wide field: fungal, bacterial, and virus diseases; resistance of plants to poisons and alkalies; the relation of heredity, of nutrition, or of various chemical substances or physiological products to resistance; phagocytosis in plants. The work is marred by irritating misprints, e.g., on p. 85 the following variants of *Phyllosticta* occur: Phylosticta, Pyllosticta, Phallosticta. The papers form an excellent cross section of our present state of knowledge and should be a stimulus to further research on this intriguing and important subject.

WILLIAM B. BRIERLEY.

The Genus Septobasidium. By JOHN N. COUCH. Pp. ix + 480. University of North Carolina Press. 1938. $ 5.0.

All known species of *Septobasidium* occur on living plants in association with scale insects, and any fungus showing such relation is of interest to applied biologists, not only intrinsically, but because it may have importance in the field of biological control. When the author commenced his studies some 12 years ago about 75 species of *Septobasidium* had been named, but few had been described adequately or illustrated. In the intervening period Prof. Couch has added greatly to our knowledge of the genus and he has now assembled his studies in this imposing volume.

Ch. I (pp. 44) is a convincing and interesting account of the fungus-insect relationship based on the author's study of *S. Burtii* and certain other species. He concludes that "The fungus and insects live together interdependently; the fungus furnishes a home and protection for the insects, while, in return, the insects furnish food and a means of distribution for the fungus. . . . The relationship here is, therefore, obviously one of symbiosis; both fungus and insects are benefited by the association at the expense of the tree." The author suggests that the form of the fungus is due to its response to a stimulus from the insects and is, perhaps, analogous to a "gall development". This chapter raises interesting problems for further study, e.g. why, when grown on artificial culture media, inappropriate living larvae, dead insects, etc., the fungus does not fruit or show any of the striking features so characteristic of its growth on the living scale insect.

In Ch. II (pp. 3), dealing briefly with pathological considerations and methods of control, the author concludes that although the damage to the trees may not be serious, "it is imperative that we look upon the *Septobasidium*-scale insect combination as distinctly harmful". Smearing kerosene emulsion paste on the patches of *Septobasidium* gave good results, but pruning of infected branches during the dormant season is regarded as the most effective means of control.

Ch. III (pp. 8) is devoted to the geographical distribution, host trees, and host insects of the fungus; Ch. IV (pp. 5) to structural features of taxonomic importance; Ch. V (p. 1) to hybridization in *Septobasidium*; and Ch. VI (p. 1) to its cytology. In nature the fungus seems rather prone to hybridization and as, apparently, it grows readily *in vitro* it would, could it be persuaded to reproduce, be a very interesting subject for genetical study. In Ch. VII (pp. 2), dealing with the relationships of the fungus, the author regards it "as a separate order on a par with the Auriculariales, Uredinales, and Ustilaginales". In Ch. VIII (pp. 2) the genus *Septobasidium* is described, and Ch. IX (pp. 10) contains an excellent key to the species. Ch. X (pp. 219) gives detailed diagnoses in English and descriptions of 170 species, and notes on three incompletely known species and on three excluded species.

The book is illustrated by a frontispiece, 60 text-figures, and a magnificent series of 114 plates. Sixty-six of these contain original drawings of microscopical features and the remainder original photographs of the fungus on host trees. There is a bibliography of 120 citations, and an index.

The work is a masterly production and ranks among the notable volumes in mycological literature.

WILLIAM B. BRIERLEY.

Soilless Growth of Plants. By CARLETON ELLIS and MILLER W. SWANEY. Pp. 155. 58 figs. New York: Reinhold Publishing Corp.; London: Chapman and Hall. $ 2.75 (13*s.* 6*d.*) net.

There is nothing new to botanists in the idea of growing plants without soil in nutrient solutions; many different methods employing water or sand culture have been used in scientific investigation of plant nutrition. It is, however, only in recent years that the possibility of using any of these methods for large-scale production of crops has been worked upon, principally in the United States. Two main lines have been followed: at Purdue University and the New Jersey Agricultural Experiment Station the growing of plants in mineral aggregates (sand, gravel, cinders or other material) has been tried on a commercial scale and found to give results with many crops at least as good as the normal methods of cultivation. In California, the work under Gericke has been directed to the commercial exploitation of liquid-culture methods, the so-called "tank culture". Reports of the successes achieved, especially in California, caught the fancy of journalists of the popular press, first in America and then in this country, and wild and fantastic stories were published of crop yields 400 and 500% above normal.

The truth is, of course, that the methods may well have commercial advantages, but these lie, not so much in greatly increased yields, as in the greater degree of control over the nutrition of the crop and the presumably greater freedom from, or at least ease of control of, soil-borne diseases. The problem almost certainly is principally one of comparative cost, but in these days of need for expansion in food-producing power the idea is one which must be investigated.

The book under review is a clear and impartial account of the various methods which have been tried, or which might be tried, by the large-scale producer or the amateur. Full details with illustrations are given of the different types of apparatus and a range of suggested nutrient solutions is appended. An interesting chapter deals with the uses of the synthetic growth-substances and the chemical colchicine, recently shown by Blakeslee to have the remarkable property of inducing polyploidy in many plants. The book is one which should be read, not only by those interested in the practical problem, but by all workers on plant nutrition. They will find practical hints of great value.

R. H. STOUGHTON.

Plant Growth Substances. By HUGH NICOL. Pp. xii + 108. London: Leonard Hill, Ltd. 1938. 3*s.* 6*d.* net.

The chief difficulty in reviewing this book lies in understanding to whom it is addressed. It is based upon a series of articles contributed by its author to *The Manufacturing Chemist* in 1937, from which one would infer that it was written mainly for chemists concerned either in the preparation or the identification and analysis of the substances. Certainly much more than half of the book would be unintelligible to anyone with no knowledge of organic chemistry. On the other hand chapters "for the layman" are introduced to give some idea of the practical uses to which synthetic growth substances have been, or may be, put. Unfortunately, for the chemist the chemistry is insufficient; to him the most valuable part will be the well-selected lists of references. The author admits frankly that he is not a physiologist, and the research worker in the field of plant response will seek in vain for the physiological implications and explanations of "hormone" action. For him, the chemical treatment will be of considerable value.

The author has, however, undoubtedly rendered service in collecting together so much information on the occurrence, sources, synthesis and identification of these substances, and in presenting it at a price so low that there can be no excuse for anyone who is even remotely interested in the subject not availing himself of that information.

It is unfortunate that, on p. 11, the author states that "at present...the only commercially available growth-substances the horticulturist and amateur gardener need trouble about, are *indole-acetic acid* and *phenyl-acetic acid*". Naphthalene-acetic acid and indole-butyric acid, as well as others, have been available for some time, certainly since before this book was written. The latter, at least, is probably a more useful compound than either of those mentioned, while the former is the most potent stimulator of root-initiation although, at the same time, having the greatest bud-inhibiting power. It may be noted that a commercial firm actually advertises five synthetic substances at the back of this very volume.

The author begins, apparently, in one chapter to attempt to clear up the vexed question of what general name should be used for these substances but, after seeming to favour the claims of Bottomley's term "auximone", he allows it to be inferred elsewhere in the book that he suggests the use of "growth-regulating" substance which he attributes to Eden but which was, in fact, used by Snow ten years ago.

As a reference book and source of information on the original (chemical) literature the book will be of real value to the research worker. It is feared that the "layman" will retire with a headache.

R. H. STOUGHTON.

Common British Grasses and Legumes. By J. O. Thomas and L. J. Davies. Pp. viii + 124. London: Longmans, Green and Co., Ltd. 1938. 6*s.* 0*d.*

The importance to the agriculturist of a knowledge of the grasses is so generally recognized, that little need be said to enforce the value of another book. This book has been written for the farmer, schools, young farmers' classes and agricultural colleges and is exceptionally well illustrated. It is designed to supply, in concise form, a guide to the identification of the various species of British grasses and legumes. It is written in a simple, easy style and, for those who wish to use a technical book, it is one of the first that might be read with profit. The beginner often finds difficulty in distinguishing between the different species, but by using this book the student should learn the characters of the common forage plants with celerity. The main chapters deal with botanical descriptions of the more common grasses and legumes, arranged in alphabetical order of their species. The peculiarities of the structure of the various grasses are carefully explained and each species is illustrated by excellent line drawings. Two easily worked keys for the identification of the grasses and clovers by their vegetative characters are given. The authors have made the book intelligible to persons possessing little scientific knowledge: it also commends itself to those workers whose duty it is to educate the farming community.

J. S. L. WALDIE.

Plant Ecology. By J. E. Weaver and F. E. Clements. 2nd Edition. Pp. xxii + 601. London: McGraw-Hill Publishing Co., Ltd. 1938. 30*s.* 0*d.*

The first edition of this well-known American textbook was reviewed in the *Annals*, 1930, **17**, 398. In the intervening period the whole front of ecology has advanced, most strikingly perhaps, in methods of studying vegetation, and in knowledge of the fundamental units, of ecesis and invasion, of reactions and coactions, and in the study of the effects of light, wind and drought upon vegetation. The concept of xerophytism has greatly changed. The vast importance of climate and vegetation in soil development has been generally recognized, and the processes of plant succession, stabilization of climax vegetation, and the use of plants and plant communities as indicators, have become much clearer. These advances are reflected in the new edition which has largely been rewritten, and rearranged to follow a more logical sequence. The volume has been increased by 81 pages, the number of text-illustrations by 9 figures, and the bibliography by 430 citations. The book remains primarily American in outlook, with examples and illustrations drawn from American work and conditions, but it is one of the major landmarks in the literature of modern ecology.

WILLIAM B. BRIERLEY.

Forest Bibliography to 31st December 1933. Compiled and published by the Department of Forestry, University of Oxford. Part III, pp. 201–74. 1938. 12*s.* 6*d.*

Parts I and II of this *Bibliography* were reviewed in the *Annals*, 1938, **25**, 438. Part III covers C, Forest Protection, under the following headings: (1) Man. Demarcation, offences; (2) Animals, including bird and game preservation; (3) Atmospheric influences. Frost, insolation, wind, hail, snow, etc.; (4) Fire; (5) Weeds, including phanerogamous parasites, climbers, etc. (special, not included under Tending); (6) Other agencies: floods, swamps, shifting sands, avalanches, landslips, etc.; reclamation works, including protective afforestation; drainage; damage by chemical fumes; shelter-belts, shade trees, etc.; (7) Fencing, including hedges.

WILLIAM B. BRIERLEY.

Textbook of Dendrology. By W. M. Harlow and E. S. Harrar. Pp. xiii + 527. London: McGraw-Hill Publishing Co., Ltd. 1937. 25*s.* 0*d.*

An introductory section of 38 pages deals with nomenclature, classification, identification, variation, and description of species: a section of 193 pages is devoted to the Gymnosperms; and the remainder of the book to Angiospermous trees. There are a short glossary, 11 pages of selected references, and an index. Each plant family is characterized briefly, and a tabular conspectus of native genera given. Generic characters are described, and the several species receive consideration under the headings: distinguishing characters, general description, range, botanical features. There are 224 text-illustrations, each containing several figures, of botanical features of the more important species. The book covers the important forest trees of the United States and Canada. The American code of nomenclature is used but, where they differ, international code names are given in italics. The book is a useful compendium and, in addition to the immediate data, contains some generally interesting botanical matter and occasional notes on fungal diseases and insect pests.

WILLIAM B. BRIERLEY.

Cryptogamic Botany. By Gilbert M. Smith. Vol. I: Algae and Fungi. Pp. viii + 545. 24*s.* 0*d.* Vol. II: Bryophytes and Pteridophytes. Pp. vii + 380. 18*s.* 0*d.* London: McGraw-Hill Publishing Co., Ltd. 1938.

These two volumes contain an excellent general survey of the classification and morphology of the Cryptogams; more physiological and applied aspects are excluded. They are suitable for students reading for a general degree in botany, and the briefly annotated bibliographies, containing about 2000 references, will be useful for honours students and research workers. The consideration of the various groups is largely on the basis of representative American types and series, but most of the examples are widely spread and available to European workers. The author's expectation "that some botanists will disagree with the allocation of space, especially in the relative proportions devoted to the algae and to the fungi" is likely to be realized since the algae receive 339 pages and the fungi 147. Furthermore, the treatment of the fungi seems to me to be less satisfactory than that of the other major groups. A notable feature are the 523 rather beautiful text-illustrations many of which have been drawn specially for the book. On the other hand there are a number of rather irritating misprints, particularly in the spelling of generic and specific names. The work is a fine achievement and a valuable addition to cryptogamic literature.

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Evolution. Essays on Aspects of Evolutionary Biology presented to Prof. E. S. Goodrich on his Seventieth Birthday. Edited by G. R. de Beer. Pp. viii + 350. With a frontispiece and 2 plates. Oxford: Clarendon Press. 1938. 15*s.* 0*d.*

The presentation of a "Festschrift" by the colleagues and pupils of a distinguished man on his attainment of 70 years is a pleasing custom, and the recipient's pleasure must be increased when the volume lies in a field in which he has always been deeply interested and to which he has made notable contributions.

The Preface states that the book covers "as evenly as possible the various more important aspects of modern knowledge concerning evolution", but this is something of an exaggeration since except for J. B. S. Haldane's essay on "The nature of inter-

specific differences" and that by H. G. Thornton on "Bacterial strains and variation", plants and plant evolution are almost unmentioned. The nineteen essays are all interesting and well written, although certain of them are, perhaps, a little thin. The subjects dealt with cover a wide range, but in addition to the two already mentioned the essays which may be of interest to applied biologists are the following: "Insect adaptation as evidence of evolution by natural selection" by Sir Edward Poulton: "The genetic basis of adaptation" by E. B. Ford: "The formation of species" by O. W. Richards: "Life-cycles of certain infusoria with observations on specificity in parasitic protozoa" by Helen P. Goodrich: "Helminths and evolution" by H. A. Baylis.

The book closes with a bibliography of the scientific works of Prof. E. S. Goodrich, an index, and a list of subscribers.

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Heredity. By A. Franklin Shull. 3rd Edition. Pp. xvii + 442. London: McGraw-Hill Publishing Co., Ltd. 1938. 21*s.* 0*d.*

The chapter headings run as follows: Development of knowledge of genetics: Fundamental structure of organisms: Production of new cells: Origin of new individuals: Development of new individuals: Mechanism of heredity: Simplest phenomena of heredity: Dominance: Backcross and testcross: Sex-linkage: Multiple alleles: Lethal genetic characters: Two or more independent pairs of genes: Interaction of genes: Modified F_2 ratios: Modification by environment: Chance and heredity: Linkage: Proof that genes are in chromosomes: Non-Mendelian inheritance: Determination and development of sex: Heredity and evolution: Inheritance of human structural characters: Human heredity, physiological characters: Inheritance of mental characters: Practical applications of heredity: Eugenics: The population problem: Race problems: Immigration. There are also a statistical appendix, questions and problems on the various chapters, a selected bibliography of 269 references, and an index.

The book is a good general introduction to heredity, illustrated mostly by animal examples, and with human heredity emphasized throughout. The usual sequence in general texts on this subject is the presentation of experimental results followed by their cytological interpretation, but the author's teaching experience has led him almost to reverse this sequence. The book is a well written and balanced exposition, and even in the later chapters the author usually does not go beyond his evidence.

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